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**USE OF BIOTECHNOLOGY TO INCREASE THE CONTENT OF
BIOACTIVE COMPOUNDS IN FERMENTED FOODS OF PLANT ORIGIN**

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*For He draws up drops of water,
Which distill as rain from the mist,
Which the clouds drop down
And pour abundantly on man.
Indeed, can anyone understand the spreading of clouds,
The thunder from His canopy? (Job 36:27-29)
Also it is not good for a person to be without
knowledge(Prov 19:2)....*

CONTENTS

I DEDICATE THIS WORK.....	4
<i>ABSTRACT</i>	7
<i>PUBLICATIONS</i>	9
ABBREVIATIONS USED	11
PART I. BIOACTIVE COMPOUNDS IN CEREALS PRODUCTS AND THEIR HEALTH BENEFITS ..	12
Background	13
CHAPTER I. INTRODUCTION	15
1. WHOLE GRAIN CEREALS	16
2. CEREALS BETA GLUCANS	34
3. RESISTANT STARCH.....	41
4. FRUCTANS	53
CHAPTER II. AIMS	96
RESEARCH ARTICLE I. EFFECT OF OF KERNEL MATURATION STAGE, SOURDOUGH FERMENTATION AND BAKING TEMPERATURE ON DIETARY FIBRE AND PHENOLIC COMPOUNDS	97
RESEARCH ARTICLE II. METABOLOMIC ANALYSIS OF SOURDOUGH FERMENTED DOUGH AND BREAD OBTAINED BY DIFFERENT IMMATURE WHEAT FLOURS.....	112
CHAPTER III. INFLUENCE OF KERNEL MATURATION STAGE AND BREAD MAKING ON THE CONTENT OF RESISTANT STARCH, FRUCTANS AND β -GLUCANS.....	129
PART II. EFFECT OF WHOLE GRAIN COMPOUNDS ON THE HUMAN METABOLOME	143
CHAPTER I. INTRODUCTION	144
RESEARCH PAPER I. IMPACT OF KAMUT® KHORASAN ON GUT MICROBIOTA AND METABOLOME IN HEALTHY VOLUNTEERS.....	163
CHAPTER II. USE OF FIE-MS AND GC-tof-MS TO STUDY THE EFFECT OF WHOLE GRAINS CONSUMPTION ON THE HUMAN METABOLOME.....	190
GENERAL CONCLUSIONS	210

ALL TO JESUS-CHRIST BE THE GLORY!!!!

I DEDICATE THIS WORK

To my family Moiffo Saa Blandine, Djusse Saa Denise, Sahmo Saa Jean Bertin,
Matadzo Saa Alvine, Makuete Saa Eugenie, Fopa Saa Bruno, Saa Victor, Saa Pierre
and Noubosse Edouard

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THANKS TO YOU ALL AND MAY GOD BLESS YOU

ABSTRACT

Biotechnological processes such as fermentation and baking and agronomical practices can increase or decrease the concentration and bioavailability of bioactive compounds such as dietary fiber components and phenolic compounds on cereal whole grains. One of the principal challenges of the food industry is to find out the better combination between the various food processing to increase the concentration of these compounds in food because of their nutritional, and healthy properties for humans.

The objectives of this PhD research were therefore: i) to evaluate the use of bread making process to increase the content of β -glucans, resistant starch, fructans, dietary fibers and phenolic compounds of kamut khorasan and wheat breads made with flours obtained from kernels at different maturation stage (at milky stage and fully ripe) and ii) to study the impact of whole grains consumption in the human gut.

The fermentation and the stages of kernel development or maturation had a great impact on the amount of resistant starch, fructans and β -glucans as well as their interactions resulted highly statistically significant. The amount of fructans was high in kamut bread (2.1g/100g) at the fully ripe stage compared to wheat during industrial fermentation (baker's yeast). The sourdough increases the content of polyphenols more than industrial fermentation (straight process) especially in bread made by flour at milky stage.

From the analysis of volatile compounds it resulted that the sensors of electronic nose perceived more aromatic compound in kamut products, as well as the SPME-GC-MS, therefore we can assume that kamut is more aromatic than wheat, so using it in sourdough process can be a successful approach to improve the bread taste and flavor

Cereal whole grains consumption influences the health status of the human gut. The determination of whole grain biormakers such as alkylresorcinols and others using FIE-MS AND GC-tof-MS is a

valuable alternative for further metabolic investigations. The decrease of N-acetyl-glucosamine and 3-methyl-hexanedioic acid in kamut faecal samples suggests that kamut can have a role in modulating mucus production/degradation or even gut inflammation.

Depending on the food functionality we want to increase we used formulation and process to enhance specific bioactives such as dietary fibre or a phenolic compound. This work gives a new approach to the innovation strategies in bakery functional foods, that can help to choose the right or best combination between stages of kernel maturation-fermentation process and baking temperature.

PUBLICATIONS

- Danielle Taneyo Saa, Silvia Turrone, Diana Isabella Serrazanetti, Simone Rampelli, Simone Maccaferri, Marco Candela, Marco Severgnini, Emanuela Simonetti, Patrizia Brigidi, Andrea Gianotti. Impact of Kamut® Khorasan on gut microbiota and metabolome in healthy volunteers. Submitted to Food Research International
- Raffaella Di Silvestro, Alessandro Di Loreto, Ilaria Marotti, Sara Bosi, Valeria Bregola, Andrea Gianotti, Danielle Taneyo Saa, Giovanni Dinelli. Nutrient, dietary fibre and phenolic contents of wholemeal obtained from immature wheat grain. Submitted to Journal of Agricultural and Food Chemistry
- Balestra, A.Gianotti, D. Taneyo Saa, G.G.Pinnavaia 2013 Durum wheat and kamut® bread characteristics:Influence of chemical acidification 7th International congress FLOUR-BREAD ‘ 13. 9th Croatian Congress of Cereal Technologist Brašno-Kruh ’ 13. Opatija, Croatia, October 16-18, 2013. Pag. 39. ISSN 1848-2554 (oral presentation).
- Danielle Taneyo Saa, Simone Maccaferri, Diana Isabella Serrazanetti, Silvia Turrone, Patrizia Brigidi Andrea Gianotti (2013). Impact of Kamut® Khorasan on gut microbiota and metabolome in healthy volunteers. Foodomics, the science for discovering. 2013, Cesena May 22-24 2013. Abstract book ISBN 978-88-902152-3-0 p. 111-112.
- Danielle Laure Taneyo Saa. Use of Biotechnology to Increase the Content of Bioactive Compounds in Fermented Foods of Plant Origin. Workshop / XVII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology 2012. Proceedings book (page 261).
- A.Gianotti, D.I. Serrazanetti, and D. Taneyo Saa. Gut metabolome modulation in healthy volunteers subjected to two different cereal-based food diets/V Symposium on Sourdough, 2012. Abstract book ISBN 978-951-38-7875-7 p 127.

- D.L. Taneyo Saa, A.Gianotti. Effect of seed maturation stage and breadmaking process on the content of beta glucans, arabinoxylans and resistant starch in wheat flour bread. 5th International Dietary Fibre Conference 2012. Rome May 7-9 2012.

ABBREVIATIONS USED

AACC	American Association of Cereal Chemists
ADF	Acid Detergent Fiber
AOAC	Association of Official Analytical Chemists
BG	β -glucans
CHD	Coronary heart disease
DF	Dietary fiber
DP	Degree of Polymerisation
FDA	Food and Drug Administration
FOS	Fructo-oligosaccharide
GI	Gastro-intestinal
GI	Glycemic index
GLC	Gas Liquid chromatography
HPLC	High-performance liquid chromatography
IDF	Insoluble dietary fiber
LAB	Lactic acid bacteria
NDF	Neutral Detergent Fiber
NFC	Non-fibrous polysaccharides
NSP	Non-starch polysaccharide
OHC	Oil holding capacity
RDS	Rapidly digestible starch
RS	Resistant starch
SCFA	Short chain fatty acids
SDF	Soluble dietary fiber
SDS	Slowly digestible starch
SW	Swelling
WHC	Water holding capacity

PART I. BIOACTIVE COMPOUNDS IN CEREALS PRODUCTS AND THEIR HEALTH BENEFITS

Background

BIOACTIVE COMPOUNDS IN CEREALS

Cereals are staple foods providing major source of carbohydrates, proteins, B vitamins and minerals in our diet. Cereals contain a broad range of substances which have health promoting effects; these substances are often referred to as phytochemicals or bioactive compounds. These bioactive compounds are generally dietary fibres (arabinoxylans, β -glucans, cellulose, lignin and lignans), sterols, tocopherols, tocotrienols, alkylresorcinols, phenolic acids, vitamins and microelements

Hans-Konrad Biesalski et al. (2009) defined bioactive compounds as "essential and nonessential compounds (e.g., vitamins or polyphenols) that occur in nature, are part of the food chain, and can be shown to have an effect on human health".

Bioactive compounds are also referred to as nutraceuticals, a term (changed in 1979 by Stephan DeFelice) that reflects their presence in the human diet and their biological activity. Bioactive substances occurring as natural in foods, dietary supplements and herbal products, and have health promoting, disease preventing.

Nutraceuticals vary from isolated nutrients, dietary supplements, and diets to genetically engineered “designer” foods, herbal products, and processed foods, such as cereals, soups, and beverages. The raising of attention in nutraceuticals is due to the fact that many scientific studies had demonstrated a positive correlation between a specific diet or a component of the diet and a lower risk of chronic disease. Until relatively recently, vitamins and other micronutrients were recommended only to avoid the classic symptoms of deficiency. The application of advance modern techniques in biology have given researchers the opportunity to study and investigate more into the molecular and cellular needs of the organism. For example, clinical symptoms of deficiency can be the result of a long-term low intake of micronutrients (Hans-Konrad Biesalski et al, 2009). However, before these symptoms occur, the inadequate delivery of micronutrients to their target tissue causes alterations that may trigger the development of chronic disease. Similar effects on genetic regulation in target tissues may take place if nutrients are overdosed.

The compounds that have been studied mostly are phenolic compounds for their antioxidant properties, an increased intake of which can protect against degenerative diseases like heart diseases, reduce the risk of chronic diseases including cancer and cardiovascular disease.

An intake of folate exceeding the standard recommendation may help to protect against coronary heart disease in cases of high homocysteine in groups with the mutation.

Different classes of phytochemicals (e.g., phytoestrogens) have been identified as having a preventive effect against specific diseases, mainly at very early stages of disease development, particularly concerning cancer. The fact that these compounds occur mainly in vegetables seems to confirm the epidemiologic evidence that a high intake of vegetables is associated with a lower risk of different kinds of cancer.

At the same time, state-of-the-art technologies, including biotechnology, have led to nutritional discoveries, product innovations, and mass production on a record scale. These developments have spawned an important and dynamic new area of research, resulting in increasing numbers of nutritional products with potential medical and health benefits.

Scientific and technologic developments are increasing the possibilities of modifying traditional foods and developing new food sources to meet these newly discovered requirements. Using modern genetics, chemistry and molecular biology, the scientific community is now able to design and manufacture foods having specific characteristics.

These new products represent a major departure from traditional foods, in part because they are based on a new approach to nutrition, i.e., as having the potential to lower the risk of chronic disease. Current nutritional approaches are beginning to reflect a fundamental change in our understanding of health. Increasing knowledge of the impact of diet on regulation at the genetic and molecular levels will lead to a rethinking of health goals, resulting in dietary strategies based on aggregation rather than individual factors.

Consequently, the health benefits of phytochemicals should be evaluated using isolated compounds or well-characterized mixtures or extracts in selected and validated *in vitro* and *in vivo* systems. These include bioavailability, dose–effect levels in target tissues, and the definition of biological markers (biomarkers) that allow the examination or investigation of an effect of a phytochemical at a very early stage of disease development. As whole grain cereals are one of the major sources of bioactive compounds, in this dissertation the main focus will be on their properties and qualities of and also their health benefits on human.

CHAPTER I. INTRODUCTION

1. WHOLE GRAIN CEREALS

1.1. Whole-grain and wholemeal

Despite the term ‘whole-grain’ is widely spread, it is mostly confused with ‘wholemeal’. The general opinion thinks that whole-grain based products are made with wholemeal flour and that they may secondarily also contain intact grains. However, the form in which grain is incorporated into food, intact or milled is nutritionally significant. Thus ‘wholemeal’ (made of milled whole-grain flour) and ‘whole-grain’ (made with intact cereal grains) breads have different effects on postprandial glycaemia. David J.A. Jenkins et al. (1988) demonstrated that whole-grain breads produce a significantly lower glycaemic response than the wholemeal breads. This emphasizes the importance of food structure on human physiology. To avoid a misunderstanding the term ‘whole-grain’ should be used for cereal based food containing more or less intact cereal kernels, and ‘wholemeal’ for cereal based-food made of more or less refined flour, in which bran, germ and endosperm are first separated, and then reassembled, in proportions that rarely correspond to those of intact grains, as the germ fraction is generally removed. The official definition of whole grains has been given by the AACC in 1999.

1.2. Definition

According to the definition in 1999 of the American Association of Cereal Chemists (AACC) whole-grains consists ‘of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact caryopsis’ (AACC International, 1999). Later in May 2004, the Whole Grains Council gave another definition including processed food products: ‘Whole grains or foods made from them containing all the essential parts and naturally-occurring nutrients of the entire grain seed. If the grain has been processed (e.g. cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver approximately the same proportion of bran, germ and endosperm that are found in the original grain seed’ (EUFIC, 2009).

In 2006, the US Food and Drug Administration published a Draft Guidance on Whole-grain label Statements that adopted the international AACC definition and included amaranth, barley, buckwheat, bulgur, maize (including popcorn), millet, quinoa, rice, rye, oats, sorghum, teff, triticale, wheat and wild rice; pearled barley was not included because some outer layers of the bran fraction are removed (FDA, 2006). In that same Draft Guidance they gave the definition of cereals which are "generally considered to be the seed heads of grasses from the Poaceae (synonymous

with Gramineous) family", and pseudocereals which are "seed heads of a number of different species of plants that do not belong to the grass family and do not include legumes or oilseeds".

Pseudocereals such as amaranth, buckwheat and quinoa were included in cereals definition for they have similar macronutrient compositions (carbohydrates, proteins and lipids), and are used in the same traditional ways as cereals such as bread making, starch staples and side dishes (AACC International, 2006; Jones JM, 2008). The response to the US Food and Drug Administration Draft Guidance by the AACC International recommended that some traditional cereals that have traditionally been lightly pearled to assist in removal of their inedible hulls such as 'lightly pearled barley' (also called de-hulled barley), durum wheat (as grano), nixtimalized corn and bulgur that has been minimally processed be also considered as whole grains' (AACC International, 2006), making allowance for small losses of components (especially bran and germ) that occur through traditional processing.

The Whole Grain Task Force stated in 2008 that it 'supports the use of the term whole-grain for products of milling operations that divide the grain into germ, bran and endosperm, but then recombine the parts into their original proportions before the flour leaves the mill' (Jones JM, 2008). In late April 2013, the AACCI Board of Directors approved the Whole Grain Working Group's characterization of a whole grain product: "A whole grain food must contain 8 grams or more of whole grain per 30 grams of product."

AACCI's recommendation will aid consumers in choosing which food products eat to meet the federal government's dietary guidelines. Additionally, a standard characterization of a whole grain food also levels the playing field for everyone in the cereal grain industry and allows for uniform messaging about whole grain food products. The language does not impact statements about products that are allowed by the law, other ingredients that might be in a food product, or the naming of food products.

However, most of the products used in studies and found in retail store showing the health benefits of whole-grain cereals are made of recombined or reconstituted whole-grain flours (Jones JM, 2008), which rarely contain the same proportions of bran, germ and endosperm as the intact grain before milling. Thus, the germ fraction is almost always removed because its high lipid content (about 9%) may go rancid upon storage (Srivastava, 2007). As a consequence of processing, whole-grain cereals substantially lost essentials minerals, vitamins and bioactive compounds so they cannot really deliver 'approximately the same rich balance of nutrients that are found in the original grain seed' (EUFIC, 2009).

The main whole-grain cereals consumed worldwide are wheat, rice and maize, followed by oats, rye, barley, triticale, millet and sorghum. In general the kernel of wheat is composed of 10–14% bran, 2.5–3.0% germ and 80–85% endosperm, depending on the intensity of the milling process. The bioactive compounds are distributed within these different parts as showed in (Figure 1), and this allocation varies according to the type and variety of cereal considered. Whole-grain cereals are a rich source of dietary fibre and bioactive compounds. For example, whole-grain wheat contains about 13% dietary fibre and at least 2% of bioactive compounds that are not fibre (Table 1), which accounts for at least 15% of the whole grain. In the bran and germ fractions, still higher proportions are reached: about 45 and 18% of dietary fibre, and about 7% and at least 6% of bioactive compounds, respectively; which represents about 52% and at least 24% of these fractions. These proportions obviously depend on the cereal type (Anthony Fardet, 2010). It is therefore easy to understand that refined cereal products that lack the bran and germ fractions have lost most of their protective compounds. For example, refining wholegrain wheat may lead to the loss of about 58% of fibre, 83% of Mg, 79% of Zn, 92% of Se, 70% of nicotinic acid, 61% of folates and 79% of vitamin E (Truswell AS, 2002).

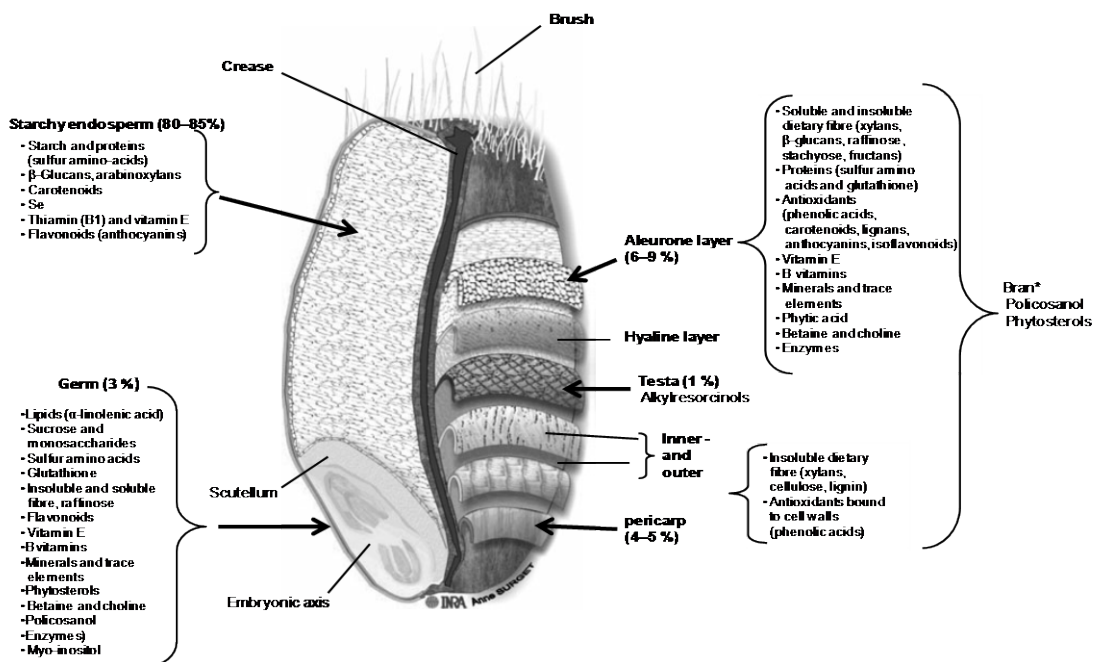


Figure 1. The three wheat fractions (bran, germ and endosperm) with their main bioactive compounds

Table 1. Average content of the major bioactive compounds in whole-grain wheat and wheat bran and germ fractions (%).

Bioactive compound	Whole-grain wheat†	Wheat bran†	Wheat germ†
α-Linolenic acid (18:3n-3)	–‡	0.16	0.53
Sulfur compounds	0.5	0.7	1.2
Total free glutathione§	0.007	0.038	0.270
Dietary fibre	13.2	44.6	17.7
Lignins	1.9	5.6	1.5
Oligosaccharides¶	1.9	3.7	10.1
Phytic acid	0.9	4.2	1.8
Minerals and trace elements	1.12	3.39	2.51
Vitamins	0.0138	0.0398	0.0394
B vitamins	0.0091	0.0303	0.0123
Vitamin E (tocopherols and tocotrienols)	0.0047	0.0095	0.0271
Carotenoids	0.00034	0.00072	–‡
Polyphenols	0.15	1.10	> 0.37
Phenolic acids	0.11	1.07	> 0.07
Flavonoids	0.037	0.028	0.300
Lignans	0.0004	0.0050	0.0005
Alkylresorcinol	0.07	0.27	–‡
Betaine	0.16	0.87	0.85
Total choline	0.12	0.17	0.24
Total free inositols (<i>myo</i> - and total <i>chiro</i> -inositols)	0.022	0.025	> 0.011
Phytosterols	0.08	0.16	0.43
Policosanols + melatonin + <i>para</i> -aminobenzoic acid	0.00341	0.00290	> 0.00186
Total	> 15.4	51.5	> 23.9
Subtotal (without dietary fibre)	> 2.2	6.9	> 6.2

* Mean percentages of bioactive compounds found in wheat bran, whole-grain wheat and wheat germ are calculated from Table 2 as follows:

% ¼ (minimum value þ maximum value)/2.

† Expressed as g/100 g food.

‡ No data found.

§ Total free glutathione is given as glutathione equivalents ¼ reduced glutathione þ (oxidised glutathione £ 2).

k Dietary fibre content is measured according to the AOAC method as such or modified (for details, see American Association of Cereal Chemists(53)).

{ Oligosaccharides include fructans, raffinose and stachyose.

As mentioned earlier, in wheat we found, dietary fibre, n-3 fatty acids, sulfur amino acids, oligosaccharides (stachyose, raffinose and fructans), lignin, minerals, trace elements, vitamins B and E, carotenoids, polyphenols, alkylresorcinols, phytic acid, betaine, total choline-containing compounds, inositols, phytosterols, policosanols and melatonin, and as each of these compounds has its own physiological and biological functions it is difficult to really define the positive effects exerted on human health by whole-grain cereal products (Adil Gani et al, 2012). In the study of the whole grain cereals compounds we have to take into account two important factors. The first is the synergy between the actions of compounds and the second is the influence of cereal matrix on the accessibility of compounds in the digestive tract and hence on their availability within the gut (Anthony Fardet, 2010). In fact, some mysteries behind the bioavailability of many bioactive compounds derived from complex cereal products are still unresolved. One of the reasons of the "unresolved mysteries" is due to the fact that the concentration of a particular compound found in whole-grain cereals is rarely the same that is available to exert a specific physiological function,

compare to the result of consuming the free compound. The intake of whole-grain cereal compounds is associated with many protective physiological mechanisms within the digestive tract (insoluble fibre can increase transit time and faecal bulking), hormonal (Zn, Se and nicotinic acid participating in hormone activation and synthesis), antioxidative (almost all micronutrients), anti-inflammatory (for example, n-3 a-linolenic acid, Cu and ferulic acid), anti-carcinogenic (almost all micronutrients), or linked to gene regulation (for example, flavonoids), cell signalling (for example, polyphenols and redox status), energy metabolism (for example, the B-complex vitamins) and effects on enzymes (for example, some minerals and trace elements) (Anthony Fardet, 2010).

1.3. A little story of Kamut® Khorasan the grain mainly part of this study

Kamut® is an ancient grain genealogically similar to modern durum wheat. Kamut wheat has an exciting history (described fully at <http://www.kamut.com/english/index.htm>). In brief, as reported by Quinn (1999), after the Second World War, a US airman claimed to have taken a handful of grain from a stone box in a tomb near Dashare, Egypt. Thirty-six kernels were given to a friend who sent them to his father, a Montana wheat farmer. According to legend, the grain was dubbed ‘King Tut's Wheat’. Soon the novelty wore off and the grain was forgotten. In 1977 a remaining jar of ‘King Tut's Wheat’ was obtained by the Quinns, another Montana wheat-farming family, who multiplied the seed and introduced the trade name ‘Kamut’—an ancient Egyptian word for wheat. In 1990, the US Department of Agriculture recognized the grain as a protected cultivar, which was given the official name ‘QK-77’. Kamut® is a trademarked wheat that has been widely promoted in Western countries as a unique grain with a unique origin and unusual health and production qualities. No rigorous experimental evidence has been published that addresses or validates these claims. Kamut was described as out-yielding spring wheats when environmental stress occurs during the growing season, but in more ideal growing seasons, its yield is at best equal to that of standard cultivars. Plant height is approximately 130 cm, with good to excellent straw strength. Grain protein content is said to be superior to that of common wheat grown under similar environments (Stallknecht et al., 1996).

The taxonomic classification of Kamut® is as unclear as its origin. It is thought to have evolved contemporaneously with the free-threshing tetraploid wheats, and is considered to be an ancient relative of modern durum wheats. In the literature it has been variously classified as *Triticum turgidum ssp. polonicum*, *T. turgidum ssp. turanicum* and *T. turgidum ssp. durum* (Stallknecht et al., 1996).

Besides its unusual history, this crop is interesting for its properties that are due to isolation from modern breeding. Kamut® has not been in contact with synthetic substances commonly used in modern breeding programs (Hammer et al. 2000). Kamut® brand wheat is of ≈ 127 cm height and has two to three times larger grains than other wheat cultivars. The grains are narrow, vitreous and flinty with a characteristic hump (Vavilov, 1951). The grain contains 20–30 % more proteins, higher levels of eight out of nine minerals, more lipids and up to 65 % more amino acids than other wheat cultivars. Alleles for prolamin, related to good pasta quality, were identified (Rodríguez-Quijano et al., 2010). Since lipids present more energy than carbohydrates, Kamut® is characterized as high-energy wheat. Kamut® products are marketed mainly through health food outlets. Due to its sweet taste, it plays a special role in bakeries as there is no need to add any sugar to pastries produced from Kamut® flour (Quinn, 1999).

In the past it was believed that Kamut® wheat did not induce as strong allergy as other wheat in patients suffering from gluten intolerance (Quinn 1999). This information was refuted by the discovery that Kamut® brand wheat causes the same allergic reactions as *T. durum* (Simonato et al. 2002). That means that, in spite of many marketing affirmations, products made of Kamut® wheat are not suitable for celiac disease patients. The most recent study on Kamut brand wheat showed that this grain protects organisms from oxidative stress better than *T. durum* (Benedetti et al. 2012). The growing of this special wheat is exclusively managed by license agreements and requires organic certification of the crop. It is grown mainly in the USA and in a limited area of Austria (Grausgruber et al. 2004).

1.4. The importance of whole-grain cereal product consumption

In Western countries' stores, we found in stores more refined products than whole-grain cereal products. The main whole-grain cereals products are breads, breakfast cereals and consumed such as brown rice or quick-cooking whole-grain barley and wheat. According to epidemiological data, the consumption of two to three servings of whole-grain cereal per day is satisfactory to obtain positive health effects (Lang R & Jebb SA, 2003). Even though whole grain dietary guidelines are specific for each country, most of them recommend an increase consumption of whole-grain cereal products (EUFIC, 2009; Lang R & Jebb SA, 2003). For example, in the USA, dietary guidelines recommended at least three servings of whole grains/day which is about 48 g of whole-grain cereals (Welsh S et al, 1994); as well as in Australia and in Denmark between six and twelve and four servings daily are recommended respectively (EUFIC, 2009). Countries whose recommend only an increase intake of cereals products with highlighting on whole-grain products without any other

mention include Canada, UK, Greece, Germany, Austria, Switzerland and Italy (EUFIC, 2009). In USA and UK, some studies demonstrated that most people consume less than one serving/day and about 30 % any, and only 0.8 to 8 % of those surveyed in the USA consumed the recommended three servings/day (Lang R & Jebb SA, 2003; Albertson AM & Tobelmann RC., 1995; Cleveland et al., 2000). On the other hand, in Scandinavian countries, consumption of whole-grain cereal products are more emphasize particularly for rye-based products (Lang R & Jebb SA, 2003). In Norway, for example, the consumption of whole-grain products is about four folds higher than in USA (NCN, 1998), but lower than in Finland, where 40 % of the population eat four or more slices of dark bread per day (Prattala et al, 2007). The low consumption of whole grain cereals in other Western countries may have some explanations. Firstly, the health benefits of whole-grain cereal are unknown to the population. Secondly, consumer's gustatory perception of whole-grain cereal products is usually associated to unpleasant taste. Thirdly, whole-grain cereal products found on retail store shelves are not well label resulting to a difficult recognition by consumers, and less widespread than refined cereals. And finally, time and money have been indicated as hurdles to eating more whole-grain cereal products(Adams JF & Engstrom A, 2000).

1.5. Whole-grain cereals as a rich source of fibre

While cereals carbohydrate is usually used as the main source of energy, they also have important physiological and nutritional properties. Based upon their digestibility in the GI (gastro-intestinal) tract, carbohydrates can be divided into two main categories: non-fibrous polysaccharides (NFC) or simple carbohydrates and non-starch polysaccharide (NSP) or structural carbohydrates. The first, usually refers as non-structural carbohydrates, includes starch, simple sugars, and fructans, is easily hydrolyzed by enzymatic reactions and absorbed in the small intestine. And the last, mostly refers as complex carbohydrates consist of cellulose, hemicelluloses, lignin, pectin and β -glucans is resistant to digestion in the small intestine and require bacterial fermentation located in the large intestine. These compounds are also important in Neutral Detergent Fibre (NDF) which includes cellulose, hemicelluloses, and lignin and in Acid Detergent Fibre (ADF) analysis consisting of cellulose and lignin. Even though, NDF and ADF analysis are usually appropriate for animal nutrition and the analysis of roughages, the resulting breakdown of carbohydrate into simple and complex carbohydrate is a good starting point to characterize dietary fibre (James M et al., 2010). According to their structure, the constituents of dietary fibre are listed in Table 2.

Table 2. Components of dietary fibre according to the American Association of Cereal Chemists (Jones, J, 2000).

Non Starch Polysaccharides and Oligosaccharides

Cellulose
Hemicelluloses
Arabinoxylans
Arabinogalactans
Polyfructoses
Inulin
Oligofructans
Galacto-oligosaccharides
Gums
Mucilages
Pectins

Analagous carbohydrates

Indigestible dextrins
Resistant maltodextrins
Resistant potato dextrins
Synthesized carbohydrates compounds
Polydextrose
Methyl cellulose
Hydroxypropylmethyl cellulose
Resistant starches

Lignin substances associated with the NSP and lignin complex

Waxes
Phytate
Cutin
Saponins
Suberin
Tannin

Over the past years, it has been difficult on agreeing to a universal definition of dietary fibre (DF), as there are many. The AACC (American Association of Cereal Chemists) gave the following definition of Dietary fibre “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. It promotes beneficial physiological effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation”(AACC, 2006). This definition includes that fraction of starch not digested in the small intestine, resistant starch (RS).

Progress has been slow on agreeing to a universal definition of dietary fibre (Howlett et al, 2010) . Another definition from EU (Commission Directive 08/100/EC Annex II) refers to fibre as

carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belonging to the following categories:

- edible carbohydrate polymers naturally occurring in the food as consumed;
- edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic, or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;
- edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.

Concerning lignin and other minor non-carbohydrates:

“The carbohydrate polymers of plant origin that meet the definition of fibre may be closely associated in the plant with lignin or other non-carbohydrate components such as phenolic compounds, waxes, saponins, phytates, cutin and phytosterols. These substances when closely associated with carbohydrate polymers of plant origin and extracted with the carbohydrate polymers for analysis of fibre may be considered as fibre. However, when separated from the carbohydrate polymers and added to a food these substances should not be considered as fibre.”

In general, fibres in whole-grain wheat vary from 9 to 17 g/100 g edible portion, which is more than vegetables fibres (< 6 g/100 g edible portion). Consequently, if most of the Western people increase their consumption of whole grain, the dietary fibre intake will raise too, from the usual 10–15 g/d to the recommended 30–35 g/d.

In wheat, the soluble fraction is lower than the insoluble. Thus, the soluble:insoluble fibre ratio is about 1:5 for whole-grain, 1:10 for bran and 1:3 for germ. Whole-grain wheat therefore provides more quantities of insoluble fibre (up to 11 g/100 g) than soluble fibre and RS (up to 22 % for certain high-amylose barley varieties (Nilsson et al, 2008)). Cereal fibre is now recognized to be beneficial for human gut health. We found a variety of fermentable carbohydrates. All fibres compounds, whether there are soluble or not, digested in the small intestine or not, have significant physiological and nutritional benefits. The nutritional properties of lignin are still poorly known (Prattala et al, 2007, Topping D, 2007). Thus, according to their content in dietary fibre, health properties, viscosity and fermentability, fibres can be classified as reported in Table 3.

Table 3. Classification of fibres based on four characteristics (Slavin., 2009).**Fibres**

Dietary Fibre	Lignin
	Cellulose
	β -glucans
	Hemicelluloses
	Pectins
	Gums
	Resistant Starch
Soluble Fibres	β -glucans
	Gums
	Wheat dextrin
	Psyllium
	Pectin
	Inulin
Fermentable Fibre	Wheat dextrin
	Pectins
	β -glucans
	Guar gum
	Inulin
Viscous Fibres	Pectins
	β -glucans
	Some gums (e.g., guar gum)
	Psyllium
Functional Fibre	Resistant dextrins
	Psyllium
	Fructooligosaccharides
	Polydextrose
	Isolated gums
	Isolated resistant starch
Insoluble Fibres	Cellulose
	Lignin
	Some pectins
	Some hemicelluloses
Non-fermentable Fibres	Cellulose
	Lignin
Non-viscous Fibres	Polydextrose
	Inulin

Therefore, the Food and Drug Administration (FDA) has approved two health claims for dietary fibre. The first claim states that, an increased consumption of dietary fibre from fruits, vegetables and whole grains contemporaneously to a decreased consumption of fats (<30% of calories), may reduce some types of cancer (FDA, 2008) —Increased consumption is defined as six or more one ounce equivalents, with three ounces derived from whole grains. A one ounce equivalent would be consistent with one slice of bread, ½ cup oatmeal or rice, or five to seven crackers.

The second claim corroborating health benefits of DF states that a decrease risk of leading to coronary heart disease (CHD) it is associated to a diet low in saturated fat (<10% of calories) and cholesterol and high in fruits, vegetables and whole grain (FDA, 2008). Mostly, a consumption of about 25 to 35 g/d of dietary fibre is considered to be an increased is considered to be approximately, of which 6 g are soluble fibre.

1.6. Structural features of dietary fibres

Dietary fibres are composed of a mix of chemical compounds. Therefore, the determination of each compound depends on its composition, because of the complex chemical nature of fibre (Elleuch et al., 2011). In the group of dietary fibre, we found non-digestible carbohydrate, such as polysaccharides: cellulose, β -glucans, hemicelluloses, gums, mucilage, pectin, inulin, resistant starch; oligosaccharides: fructo-oligosaccharides, oligofructose, polydextrose, galacto-oligosaccharides; and soybean oligosaccharides raffinose and stachyose. Cereals are the main source of cellulose, lignin and hemicelluloses, while fruits and vegetables are the major sources of pectin, gums and mucilage (Normand, Ory, & Mod, 1987). The carbohydrate residues and the nature of the bond between them defined each polysaccharide. The complexity of dietary fibres like their chemical nature, degree of polymerisation, presence of oligosaccharide and polysaccharide need an accurate evaluation of the appropriate analytic methods to use for the quantitative and qualitative measurement of dietary fibre, in food and food by-products.

1.7. Technological functionality of dietary fibres

1.7.1. General

Fibres as a food ingredient are able to provide various physiological functionalities for each technological property, as shown in Table 4.

Table 4. Technological and physiological properties of dietary fibre products (Elleuch et al., 2011).

Technological property	Physiological functionality
Water holding capacity	Laxative
Water swelling capacity	Reduction of blood cholesterol
Water retention capacity	Reduction of blood glucose
Water solubility	Reduction the risk of chronic disorder e.g. coronary heart disease, diabetes, obesity and some forms of cancer
Oil holding capacity	
Viscosity Texturizing	
Stabilizing	
Gel-forming capacity	
Antioxidant capacity	

1.7.2. Solubility

Based on their ability to form a solution when mixed with water or not, dietary fibres are classified as soluble or insoluble. Soluble dietary fibres are constituted of pectin substances, gums, mucilage, and some hemicelluloses, whereas cellulose, other types of hemicelluloses and lignin are part of the insoluble fraction (Davidson & McDonald, 1998; Roehrig, 1988; Schneeman, 1987). Solubility of polysaccharides is depending on whether their backbone can be set regularly (insoluble) or irregularly (soluble). The presence of a substitution group such as COOH or SO₂⁻⁴ increment solubility. Solubility is also influenced by temperature and ionic strength (Bertin, Rouau, & Thibault, 1988; Fleury & Lahaye, 1991; Manas et al., 1994). The technological functionality and physiological properties of dietary fibres are influenced by their solubility. (Jimenez-Escrig & Sanchez-Muniz, 2000; Roehrig, 1988). In general, soluble fibres are able to increase viscosity, and to decrease the glycemic response and plasma cholesterol (Abdul-Hamid & Luan, 2000; Olson, Gray, & Chiu 1987; Roehrig, 1988; Schneeman, 1987), whereas insoluble fibres are able to increase faecal bulk and decrease intestinal transit (Olson et al., 1987; Roehrig, 1988) because of their porosity and low density. In food processing, the soluble fraction have greater capacity to provide viscosity, ability to form gels and/or act as emulsifiers in respect of insoluble fraction, has neither bad texture nor bad taste and is easier to incorporate into processed foods and drink

1.7.3. Hydration properties and oil-binding capacity

Hydration properties of fibres can be study by measuring their water absorption, water holding and swelling capacity. Water absorption is the kinetic of water uptake determined by a Baumann apparatus (Fleury & Lahaye, 1991), it gives exhaustive information about the fibre, particularly its substrate pore volume (Guillon & Champ, 2000). Water holding capacity (WHC) is defined as the

amount of water absorbed by 1g of dry fibres under controlled conditions of temperature, time soaked, and duration and speed of centrifugation. However, WHC is influenced by losses of small amount soluble fibres occurring during measurement (Fleury & Lahaye, 1991). In general, the amount of water quantified by centrifugation is higher compared to the Baumann apparatus (Fleury & Lahaye, 1991; Weightman, Renard, Gallant, & Thibault, 1995). Swelling (SW) can be estimated by the bed volume technique, determined by swelling the fibres in water overnight, in a volumetric cylinder (Kuniak & Marchessault, 1972). The hydration properties of dietary fibres are associated to various factors such as the chemical structure of the component polysaccharides, porosity, particle size, ionic form, pH, temperature, ionic strength, type of ions in solution and stresses upon fibres. The ability of dietary fibres to retain water is strongly related to the source of the dietary fibre. Cereal derivatives have lowest affinity for water and oil. These differences are associated to the chemical properties of the fibres. As the temperature increases, their hydration properties in water increase also, probably due to the increase in fibre solubility (Fleury and Lahaye, 1991) . Modifications of the ratio of affinity/no affinity of the fibres for water are related to the change in ionic strength.

Beside their hydration properties, fibres have the capacity to hold oil. Oil holding capacity (OHC) is the amount of oil absorbed by the fibres after blending, incubation with oil and centrifugation. Oil absorption of cereal by-products like wheat bran, is related principally to the surface properties of the bran particles (Caprez, Arrigoni, Amado, & Zeukom, 1986), yet it can be also referred to the global charge density and to the hydrophilic properties of the components, e.g., alginate and fucan, of the algae (Fleury & Lahaye, 1991). WHC, SW and OHC open the door to future applications of dietary fibres as ingredients in food products. Dietary fibres with high OHC can be used as stabilizers of high fat food products and emulsions; those with high WHC may be applied as functional ingredients to avoid syneresis and modify the viscosity and texture of some formulated foods (Grigelmo-Miguel & Martina-Belloso, 1999a).

1.7.4. Antioxidant properties

Cereals structural carbohydrates possess antioxidant properties and can be used as potential new antioxidants. Fibres with high antioxidant capacities may be exploited as ingredients that aid the stabilization of fatty food products, thus improving their oxidative stability and extending their shelf life. These high-fibre products have several technological attributes (water holding capacity, water swelling capacity, water-solubility, fat binding capacity, viscosity and antioxidant properties), that support their application as ingredients by the food manufacturing.

1.8. Effects of processing on the properties of dietary fibres

The properties of DF can be influenced by food processing such as chemical, mechanical, thermal and enzymatic processing. Many examples of chemical modifications of dietary fibres are found in the literature. Partial delignification of lignocelluloses by alkaline hydrogen peroxide treatment of fibre used to improve its functional and sensory properties by reduction of the dark colour and lignin content enzymatic treatment and extrusion are among the processes that improve the functionality of fibre (Larrauri, 1999). Chemical processes with acidic or basic solutions of sugar beet fibres enhance the SW resulted from the destruction of the coherence of the cell walls (Bertin et al., 1988). Mechanical treatment, like stirring, opens the fibre structure by mechanical shear, resulting to the availability of free hydroxyl group's cellulose available to be bind with water (Sangnark & Noomhorm, 2004).

Grinding can modify fibre properties, by damaging the regions of potential water holding capacity and, thus, decreasing the capacity to hold water. On the other hand, it can improve these properties as a result of the raise in surface area. Grinding can also increase and decrease the hydration properties of the same material, depending in its particle size.

Enzymatic processes can modify the ratio between soluble (SDF) and insoluble fibres (IDF), for example a treatment of cell walls with xylanase increases the amount of soluble dietary fibres (Laurikainen, Harkonen, Autio, & Poutanen, 1998). Thermal process can modify the ratio between insoluble–soluble fibres, total dietary fibre content, and their physicochemical properties. These modifications are influenced by the type of plant material and the nature of the process. Wet heat treatments of wheat bran affect the surface properties of dietary fibres, caused the increase of OHC (Caprez et al. 1986). Extrusion cooking, a process during which a cereal product usually, is heated under pressure, then extruded through fine pores while the super-heated water evaporates speedily producing a textured product, can enhance the total dietary fibre content (Elleuch et al., 2011). The extrusion process augments soluble dietary fibres, with formation of additional elements by transglucosidation, whereby 1,4 carbon–oxygen bonds are splitted and new anhydroglucose linkages are made. The amount of insoluble dietary fibres may increase during the formation of resistant starch type 3 (RS3), which is insoluble at room temperature (Stojceska, Ainsworth, Plunkett, & Ibanog̃lu, 2010; Vasanthan, Gaosong, Yeung, & Jihong, 2002). This process takes part when the amylose chains combine together in helical and double helical coils. Retrogradation, a process of deterioration, a reversal or retrogression to a simpler physical form, modify the quality of

the food. The pressure cooking decreases the cellulose and hemicelluloses content by breaking down polysaccharides into simple sugars (Redman, Islam, and Shah, 2003).

1.9. Analytic methods for studying dietary fibres

1.9.1. General

There is not a particular analytical method which meets all the requirements for the nutritional or chemical constituents of dietary fibre in foods (Elleuch et al., 2011). Analytical methods and techniques continue to be developed and, whether this results from modifications in the goals of the analysis or improvements in the accuracy, precision, rapidity, ruggedness and cost effectiveness of the method. We can classify the methods for the determination of dietary fibre into three main categories: non-enzymatic-gravimetric, enzymatic-gravimetric, and enzymatic-chemical methods. The later incorporates enzymatic-colorimetric and enzymatic-chromatographic (GLC/ HPLC) methods. At the moment, the official methods for the determination of dietary fibre come from the Association of Official Analytical Chemists (AOAC) ; these are the enzymatic-gravimetric (Prosky, Asp, Schweitzer, Decries, & Farad, 1988) and enzymatic-chemical method (Enlist, Quigley, & Hudson, 1994).

1.9.2. Non enzymatic-gravimetric methods

These methods are the oldest, and consist of crude fibre, acid detergent fibre and neutral detergent fibre. Although, they do not quantify water-soluble components, the dietary fibre content is underestimating (Southgate, Hudson, & Englyst, 1978). Crude fibre is constituted of the residue that remains after chemical breakdown occurring during the hydrolytic or oxidative reactions. Van Soest (1963) advanced the procedure for determining fibre by the acid detergent fibre method.

1.9.3. Enzymatic-gravimetric method

Schaller (1976) added amylase treatment into the neutral detergent fibre method (Van Soest & Wine, 1967), in order to resolve the interference occurring when the method was applied to starchy foods. Prosky et al. (1988) developed an enzymatic-gravimetric method for dietary fibre determination, resulting in the AOAC Prosky method, based on the work of Asp (1978).

These methods measure total dietary fibre (TDF) as the sum of soluble and insoluble polysaccharides and lignin (Van Soest & Wine, 1967). More details of this method can be found in section 45.4.07 of the AOAC International Official Methods of Analysis. Later on, the procedure included also the determination of insoluble dietary fibre (IDF) (32.1.16 AOAC) and soluble dietary

fibre (SDF) (45.4.08 AOAC) (Association of Official Analytical Chemists, 2000; Lee, Prosky, & DeVries, 1992). All three methods use the similar basic enzymatic-gravimetric procedure with phosphate buffer. An additional method to measure TDF, IDF and SDF was expanded in the early 1990s and is detailed in 32.1.17 AOAC. It is the same as the first method, in that it uses the same three enzymes (heat stable α -amylase, protease, and amyloglucosidase) and similar incubation conditions but replaces 2-(N-morpholino) ethanesulfonic acid-tris (hydroxymethyl) aminomethane (MES-TRIS) buffer with the phosphate buffer. The outcomes using the MES-TRIS buffer for determination of dietary fibre are the same as those obtained using the phosphate buffer. The important steps in this present gravimetric method include enzymatic treatments for starch and protein subtraction, precipitation of soluble dietary fibre components by aqueous ethanol, filtration and weighing of the dietary fibre residue, and correction for protein and ash in the residue (Prosky et al., 1988). This enzymatic-gravimetric method measures polysaccharides, lignin, some type of resistant starch and other associated compounds (waxes, phenolic compounds, Maillard reaction products) while oligosaccharide and other types of resistant starch are not quantified by this method. Ohkuma, Matsuda, Katta, and Tsuji (2000) and Gordon and Okuma (2002) included the determination of indigestible oligosaccharides into the AOAC method (Lee et al., 1992; Prosky et al., 1988), based on the determination of soluble and insoluble fibre. The soluble fibre fraction is composed of high molecular weight compounds. The filtrate from the ethanol precipitation is measured by liquid chromatography to determine the low molecular weight soluble fibre (oligosaccharide). In 2010, McCleary et al. developed a new method based on the AOAC Official Methods: 985.29 (Prosky, Asp, Scheweizer, de Vries, & Furda, 1992), 991.43 (Lee et al., 1992), 2001.03 (Gordon & Okuma, 2002) and 2002.02 (McCleary & Monaghan, 2002), which take into account the determination of non-digestible oligosaccharides and resistant starch in the high molecular weight insoluble and soluble fibre. The analytical scheme for this procedure is summarized in Figure 2.

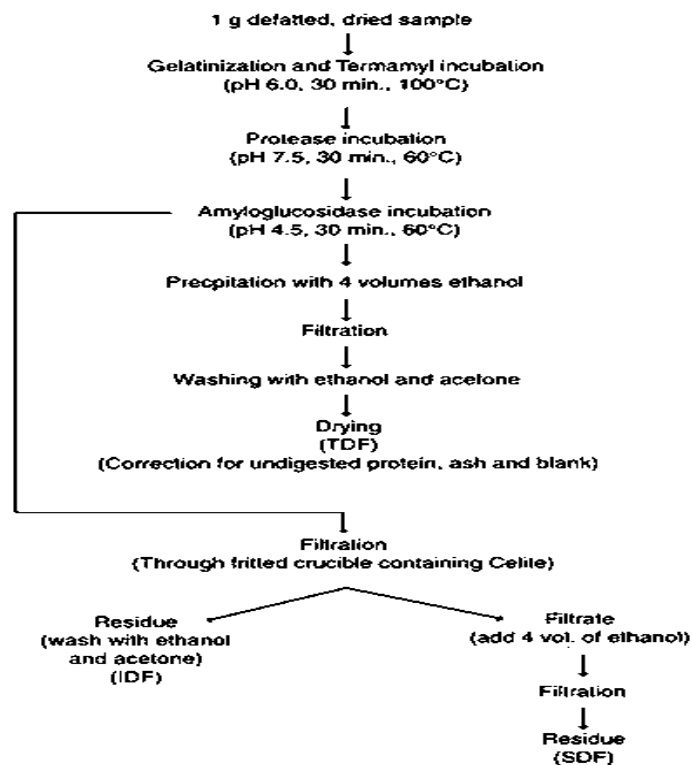


Figure 2. Dietary analysis according to AOAC Official Methods: 985.29

1.9.4. Enzymatic-chemical methods

These methods originated from the principles laid down by Southgate in 1969 (Englyst et al., 1994). They measure dietary fibre calculated as non-starch polysaccharide. Some key steps here are the enzymatic removal of starch, and sometimes protein also. From the aqueous ethanol precipitation with or without dialysis we obtain separation of the low-molecular-weight soluble dietary fibre polysaccharides from starch hydrolysis products (Englyst et al., 1994; Manas, Bravo, & Saura-Calixto, 1994). Usually, the dialysis separation is choosing to ethanol precipitation, to avoid soluble fibre loss (Manas et al., 1994). The neutral components content of the hydrolysed polysaccharides are measured in gas-liquid chromatography (GLC) or high-performance liquid chromatography (HPLC), obtaining the amount of each monosaccharide and uronic acids that are determined colourimetrically (Englyst et al., 1994). We can also use the spectrophotometry (Englyst et al., 1994) to quantify the total sugars. After the hydrolysis of total or insoluble polysaccharides, the remain residue is filtrated and quantified as Klason lignin. The sum of non-starch polysaccharide and Klason lignin are mixed together to quantify total dietary fibre. Insoluble and soluble dietary fibres can also be calculated. The determination of neutral sugars, uronic acids residues and Klason lignin may also be measured by the 'Uppsala method' (Theander, Aman, Westerlund, Andersson, &

Pettersson, 1995). Starch is removed enzymatically, and total fibres (insoluble and soluble) are precipitated with 80% and centrifuged. The precipitated are hydrolysed by sulphuric acid, and the released neutral sugars are then quantified by GLC. Uronic acids are determined by colourimetric method and Klason lignin gravimetrically. The Klason enzymatic-chemical method (Englyst et al., 1994)(54) measures only the non-starch polysaccharides and lignin. Oligosaccharide (Degree of Polymerisation or DP < 12) and resistant starch are not quantifiable, because of the use of 80% ethanol for polysaccharide precipitation and dimethyl sulfoxide (DMSO) aimed to solubilise total (resistant and not resistant) starch. Therefore, total starch is completely absorbed during these steps (Englyst, Quigley, Hudson, & Cummings, 1992). However, during the hydrolysis and/or derivatisation when GLC is used for determination of dietary fibre, there is loss of polysaccharides resulting in an underestimation of the amount of fibre (Elleuch et al., 2008).

The results obtained using these three different methods: gravimetric-enzymatic AOAC (Lee et al., 1992; Prosky et al., 1988) enzymatic-chemical (Englyst et al., 1994) and enzymatic-chemical with Klason, are different. In general, the gravimetric-enzymatic AOAC method evaluates the highest fibre content and the enzymatic-chemical method estimates the lowest. Beside these methods described above, specific methods of dietary fibre determination have been developed for the measurement of oligosaccharides (DP < 12: fructo-oligosaccharide, galacto-oligosaccharide, polydextrose) (Gordon & Okuma, 2002; Ohkuma et al., 2000; Quemener, Thibault, & Coussement, 1994), resistant starch (Craig, Holden, & Khaled, 2000; Goni, Garcia-Diz, Manas, & Saura-Calixto, 1996; McCleary & Monaghan, 2002) and related polyphenols (Goni, Diaz-Rubio, Perez-Jimenez, & Saura-Calixto, 2009). Concerning the related polyphenols, Goni et al. (2009) observed that polyphenols are the main components of insoluble dietary fibre in plant foods and the same as of soluble dietary fibre in common beverages. In the next chapters I will focus on some of the most important component of dietary fibres:

- β -glucans (BG),
- fructans
- resistant starch (RS).

2. CEREALS BETA GLUCANS

2.1. Introduction

Mixed-linkage (1→3),(1→4) linear β -D-glucans commonly known as β -glucans (BG) are mainly found on endosperm cell walls of commercially important cereals such as barley, oat, wheat, rye, sorghum, and rice. Cereal β -glucans are linear homopolysaccharides composed of D-glucopyranosyl residues (GlcP) linked via a mixture of β -(1→3) and β -(1→4) linkages. The structure features mostly the presence of two or three consecutive (1→4)-linked β -D-glucose in blocks (i.e., oligomeric cellulose segments) that are separated by a single (1→3) linkage. Even though most of the cellulose segments are trimers and tetramers, there are also present longer cellulosic oligosaccharides in the polymeric chains (Dais, P. and Perlin, 1982; Woodward et al, 1983; Woodward et al, 1988; Varum, K.M. and Smidsrod, O, 1988; Wood, P.J., 1991; Izydorczyk et al, 1998). Cereal β -glucans vary based upon their molecular/structural properties, such as molecular size, ratio of tri- to tetramers, amount of longer cellulosic oligomers, and ratio of β -(1→4)/-(1→3) linkages. The molecular features of β -glucans are an important factor of their physical properties, such as water solubility, dispersibility, viscosity, and gelation properties, as well as of their physiological function in the gastrointestinal tract. The physical and physiological properties of β -glucans are of industrial and nutritional value. Increasing interests in β -glucans during the last two decades are largely due to their acceptance as functional, bioactive ingredients. Cereal β -glucans have been associated with the reduction of plasma cholesterol and a better control of postprandial blood serum glucose and insulin responses in humans and animals (Klopfenstein, C.F, 1988; Braaten, J.T. et al, 1991, Braaten, J.T. et al, 1994; Wood, P.J. et al, 1994; Bhatt, R.S, 2003). The effect of oat and barley β -glucans in reducing the risk of coronary heart disease (CHD) has been recognized by the U.S. Food and Drug Administration (FDA), and now the EFSA approves two health claims on both oat- and barley-based foods. The potential use of β -glucans as food hydrocolloids has also been proposed based on their rheological characteristics. In addition to their importance for solution viscosity, β -glucans have been shown to gel under certain conditions (Lazaridou, A et al, 2004; Lazaridou, A. and Biliaderis, C.G, 2004; Vaikousi, H. and Biliaderis, C.G, 2005; Vaikousi, H. et al, C.G, 2004). β -Glucans can be utilized as thickening agents to modify the texture and rheology in several food formulations or may be used as fat mimetic in the development of new calorie-reduced foods. Moreover, lately there have been many efforts to increase the amount of cereal β -glucans in food formulations because of the beneficial physiological function of these polysaccharides. As a result, β -glucans-rich fractions from cereal or purified β -

glucans have been successfully incorporated into food products, such as breakfast cereals, pasta, noodles, and baked goods (bread, muffins), as well as dairy and meat products (Bhatti, R.S, 1986; Wood, P.J, 1986; Knuckles, B.E et al, 1997; Newman, R.K et al, 1998; Marconi, E et al, 2000; Cavallero, A et al, 2002). The physical properties of β -glucans, such as solubility and rheological attributes in solution and gel state, are usually controlled by linkage patterns, conformation, and molecular weight or molecular weight distributions.(Izydorczyk et al, 1998; Vaikousi, H. et al, C.G, 2004; Tosh, S.M, 2004). However, the interaction between structural features and physiological responses has been only partially explored (Wood, P.J. et al, 1994).

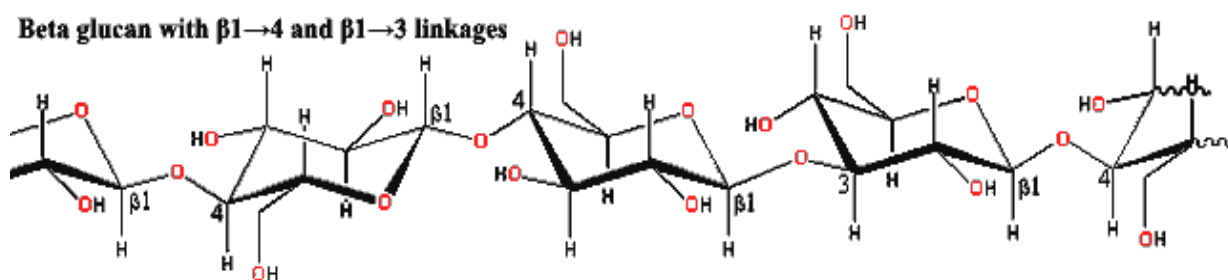


Figure 3. Beta-glucans linkages.

2.2. Occurrence

Barley and oat are recognized to be most important sources of β -glucans. The content of β -glucans in barley grain range from 2.5 to 11.3% by weight of the kernel, but they usually fall between 4 and 7%(Wood, P.J., 1991; Cavallero, A et al, 2002). The level of b-glucans in oats (2.2–7.8%), rye (1.2–2.0%), and wheat (0.4–1.4%) may also vary substantially, but it is generally lower than in barley (Lazaridou et al.,2007). However, the amount of β -glucans in barley and oat is influenced by both genetic and environmental factors and the interactions between the two (Andersson, Elfverson, Andersson, Regner, & Åman,1999). Nevertheless, some studies shown that the genetic factor of oat and barley is considered to be far more important than environmental conditions as a determining factor of the final β -glucans content of these grains. In fact, many studies highlighted that, barley genotypes with abnormal starch composition like waxy or high amylose have higher content of β -glucans than those with normal starch (Newman, R.K. et al, 1992; Wu, Y.V et al, 1994; MacGregor, A.W. and Fincher, G.B, 1993; Jadhav, S.J et al, 1998). It has been demonstrated that waxy barley has significantly higher β -glucans content than non-waxy; the combination of two recessive genes for waxy and hulless in barley leads to an increase of β -glucans levels, by approximately 1.5 to 2 times. However, there is more impact on the β -glucans content of barley when it is associated with a locus on chromosome 2(2H) (Bhatti, R.S, 2003). It has been reported that feed and six-row barleys may have slightly lower β -glucan levels than malting and other two-row varieties (MacGregor,

A.W. and Fincher, G.B, 1993; Jadhav, S.J et al, 1998). However, Fastnaught et al. (1996) have found no difference at β -glucan levels between two-row and six-row head type for any of the traits analyzed. No significant differences between hulled and hull-less barley types with normal starch characteristics were found probably because there is no β -glucans in the hull, and thus, it would be expected a change based only on the measurement being with or without 11% hull. Significant differences have been observed between 18 species of *Avena*, including nine diploids, four tetraploids, and five hexaploids grown in one location, species, and between groups based on ploidy (Miller, S.S et al, 1993). The β -glucans content in the whole grain for Finnish oat cultivars has been found to be higher for the naked oats than for the hulled cultivars (Wood, P.J. et al., 2003). The recent heightened interest and consumption of barley in human diets as a result of its high dietary fibre content could lead to an increase demand in the future for barleys with high β -glucans contents. An examination of the carbohydrate content of wild barley (*Hordeum spontaneum*) lines showed β -glucans contents ranging up to 13.2%. Thus, certain wild barley lines high in β -glucans content may represent an important genetic resource for future development of breeding programs (MacGregor, A.W. and Fincher, G.B, 1993; Wood, P.J. et al., 2003). The principal environmental factor that influences β -glucans content seems to be the availability of water during grain maturation. It has been shown that dry conditions before harvest end with high β -glucans content, and the reverse is true with moisture conditions; a functional role for the β -glucans in preventing grain dehydration may be required (Fastnaught, C.E et al, 1996; Colleoni-Sirghie, M. et al, 2004). Besides, it has been established that higher temperature during the growing time is associated to an increase in β -glucans content in barley and oat grain (Saastamoinen, M et al, 1992). It has also been found that β -glucans content had significant positive correlations with grain yield, growing time, and seed size, and significant negative correlations with protein content and hull content (Saastamoinen, M et al, 1992). The localization of β -glucans in cereal grains is important for the isolation and purification process, which aims at fractions /preparations enriched in β -glucans. Compositional analysis of isolated endosperm and aleuronic cell walls of barley indicates considerable differences between these cell groups. The endosperm cell walls are mainly composed of β -glucans (70 to 75%),(Fincher, G.B, 1975; Balance, G.M. and Manners, D.J, 1978) whereas aleuronic cell walls contain lesser amounts of β -glucans (26%). The localization of β -glucans in oat is similar to barley but the amount in cell was higher (85%). On the other hand, in wheat and rye aleurone and endosperm cell walls the main constituent are arabinoxylans. The wheat aleurone cell walls consist of 30% β -glucans, while endosperm cell walls have around 5% of β -glucans(Mares, D.J. and Stone, B.A, 1973). Some studies suggested that, differences in β -glucans amount are

strictly related to cell size and wall thickness in the starchy endosperm of oat and barley kernels (Miller, S.S. and Fulcher, R.G, 1994; Bhatta, R.S, 1991). The distribution of β -glucans in oat kernel has been showed to change according to low- and high- β -glucans varieties using the microspectrofluorometric imaging (Miller, S.S. and Fulcher, R.G, 1994). In wheat, the highest concentration is in the subaleurone layer, with little in the rest of the endosperm. In rye, β -glucans seems to be evenly distributed throughout the grain according to Parkkonen et al.(1994), whereas Harkonen et al.(1997) found that over 70% of β -glucans were concentrated in the outer fractions (bran and shorts) of a rye cultivar fractionated by a laboratory-scale roller mill.

The average of β -glucans content in whole grain was 0.6% for wheat, 4.2% for barley, 3.9% for oats, and 2.5% for rye, whereas the respective values in the endosperm from these grains were 0.3, 4.1, 1.8, and 1.7%; these analyses were applied directly to the grain by hand dissection with a scalpel without prior fractionation with a roller mill (Henry, R.J, 1987).

2.3. Extractability

Even though, the health beneficial properties of β -glucans are accountable to the soluble part of β -glucans, a high content reflects high dietary fibre content in cereal grains. In various studies it has been found that solubility is influenced by genotypic factors. The solubility of β -glucans is influenced by genotypic factors. It has been found that oats soluble β -glucans it seems to be higher than those from barley. In fact, some evidence shown that when the water-soluble β -glucans (%w/w of total β -glucans) are extracted at 38°C their range vary from oats and barley between 65 to 90% for oat and 8 to 71% for barley (Aman, P. and Graham, H, 1987; Lee, C.J, 1997). The amount of soluble β -glucans in high-amylose barley both raw and covered which gives an approximation of the extractability of β -glucans appears to be moderately low in contrast to the normal, zero, and waxy barley genotypes; consequently high-amylose barley cultivars, should not be the best font of soluble polysaccharides. Likewise, a small amount of soluble β -glucans have been found in hulless, waxy, short-awn barley genotypes despite that are often related with increased of total β -glucans (Andersson, A.A.M. et al, 1999; Andersson, A.A.M. et al, 2000). Unlike hulless barley genotypes, Waxy varieties are better source of water-soluble β -glucans. The extraction parameters (type, pH, and ionic strength of solvent, temperature, duration of extraction, and liquid–solids ratio), pre-treatment processes(heating and drying), presence of enzymes (endogenous or exogenous), likewise the grinding method and particle size; have an influence on the extractability of β -glucans (Wood, P.J, 1993; Wood, P.J et al, 1978). For example, a raise of temperature with reduction of particle size improves extraction efficiency. To have a complete extraction of β -glucans from cereal grains, it

might be necessary to use alkali or dilute perchloric or sulphuric acids though they are unsuitable for potential food uses of the isolates. We can have an enhancement of percentage of β -glucans extracted from 80 to 100%, extracting with sodium hydroxide (NaOH) yet in most cases leads to a decrease molecular size (Bhatty, R.S, 1995). In spite of the thin endosperm cell walls, wheat β -glucans are particularly resistant to extraction (Wood, P.J, 1993). The variations in proportion of β -(1 \rightarrow 3) and β -(1 \rightarrow 4) linkages in the polymeric chains, in linkage sequence on the chain, or in the degree of polymerization can explained the raise up in extractability of β -glucans when temperature or ionic strength of the solvent increase, thereby increasing physical intermolecular associations (Cui, W et al, 2000; Storsley, J.M. et al, 2003). It has been demonstrated that xylanase, arabinofuranosidase, xyloacetylsterase, and feruloyl esterase can solubilise β -glucans from the cell walls of barley endosperm, which indicates that pentosans can restrict the extraction of β -glucans (Bamforth, C.W. and Kanauchi, M.K, 2001; Kanauchi, M. and Bamforth, C.W, 2001). The presence of β -glucans that are not water extractable could be attributed to physical entrapment of β -glucans within a pentosan cross linked network via phenolic groups in the cell wall. Therefore, differences in extractability seem to be the result of features in microstructure and cell wall organization of the cereal endosperm.

2.4. Applications in Food Systems

The acknowledgment of the health benefits of cereal β -glucans resulted to an increase of application of cereal β -glucans as a functional, bioactive ingredient over the last two decades in food formulations. Many studies have been done on β -glucans enriched cereal-based foods, like breakfast cereals, pasta, and baked goods (bread, muffins); likewise, their application as thickening agents, stabilizers, and fat mimetics to modify the texture and rheology in calorie-reduced, low-fat foods has been investigated.

2.5. Cereal-Based Foods

The potential nutritional properties of β -glucans in food systems have been demonstrated by various studies using different kind of cereal food commodities (Brennan & Cleary, 2005). Oatmeal, oat flakes (rolled oats), and oat bran are usually found in breakfast hot cereals, granola cereals and bars, as well as oat flour is commonly used as ingredient in cold cereals. The high fat content of oat flour causes oxidative rancidity problems, thus a limiting use in highly expanded extruded cereal products (Webster, F.H, 1986). On the other hand, barley is an excellent ingredient for extrusion into ready-to-eat (RTE) cereal products (Newman, R.K. and Newman, C.W et al, 1991; Berglund,

P.T et al, 1994). Consumer sensory panellists ranked cereals extruded from the four 50:50 barley–rice mix and the 65:35 Wanubet barley–rice mix higher than the 100% rice cereal for crispness and colour, and ranked them comparable to the rice cereal for flavour and overall acceptability; these products contained > 2% (w/w) β -glucans. Hallfrisch and Behall (1997) reported that an oat β -glucans concentrate ('Oatprime') decreases glycaemic responses in men and women. In 2003, Hallfrisch et al. (2003) evaluated the use of β -glucans isolated from barley ('NutrimXe') and oats, and their corresponding effects on plasma glucose and insulin responses in non diabetic adults, concluding that barley β -glucans were more efficient in the regulation of glucose and insulin responses compared to oat β -glucans. Pasta is one extruded cereal product to which β -glucans have been successfully incorporated as a functional ingredient. Yokoyama et al. (1997) evaluated blood glucose and insulin responses of healthy individuals following the consumption of a control durum wheat pasta (100 g of available carbohydrate and 5 g of total dietary fibre) to that of a pasta sample with added barley β -glucans (100 g available carbohydrate, 30 g of dietary fibre and 12 g of β -glucans). Postprandial blood glucose and insulin responses decreased significantly following consumption of the pasta enriched with barley flour added to durum wheat flour. The authors attributed this drop in the glycaemic response to the incorporation of β -glucans. Similar results have been described by Knuckles et al. (1997b). Probably, the drop in glycaemic response is related to both the higher β -glucans, and increased total dietary fibre content of the experimental pasta samples. Nevertheless, further investigation have to been done to assert the exact role of β -glucans in pasta. β -glucans have also been used in other cereal based food systems such as bread. Cavallero et al.(2002) incorporated barley β -glucans rich fractions into wheat bread, and from that study, concluded that the level of β -glucans in the bread (particularly the increased soluble β -glucans level) was responsible for the diminution in glycaemic index, and that was not a result from impaired food degradation and amylolysis, but through the effect of β -glucans on digesta viscosity and glucose absorption. Likewise, in another study (Symons and Brennan, 2004b), significant decrease in starch degradation and sugar release have been found proportional to the amount of β -glucans included into the breads. Since the procedure used in that study was not dependent on glucose absorption, it seemed that the glycaemic reducing effect of β -glucans can also be related to the method used to incorporated β -glucans into the structure of the bread and may avoid starch swelling and consequently susceptibility to enzymatic degradation. The ability of β -glucans to control the rate of starch degradation and thus the glycaemic index of foods products has evident benefits with regard to obesity and diabetes. Jenkins et al. (2002) observed the reduction of glycaemic index by high levels of β -glucans fibre in two functional foods tested in type 2 diabetic

outpatients. Therefore, blood glucose levels of diabetic and pre-diabetic individuals can be moderated by using β -glucans enriched foods.

3. RESISTANT STARCH

3.1. Introduction

From the early years of emergence of nutritional science, it has been recognized that the ingested during food processing, derivatization of nutrients and creation of cross linkages take place, resulting in unavailability of food for digestion or/and metabolism (Erbersdobler 1989).

Starch is the principal source of carbohydrate in the human diet (Ratnayake & Jackson, 2008). The acknowledgment of partial digestion and absorption of starch in the small intestine as a normal fact has increased interest in nondigestible starch fractions (Cummings and Englyst 1991; Englyst and others 1992). These are called “resistant starches,” and various studies have demonstrated that they have the same physiological functions as dietary fibre (Asp 1994; Eerlingen and Delcour 1995). The variety of the current food industry and the huge variety of food products it manufactures require starches that can tolerate a broad range of processing techniques and preparation conditions (Visser et al, 1997). These needs are satisfied by modifying original starches with chemical, physical, and enzymatic methods (Betancur & Chel, 1997), which can result in to the formation of indigestible fractions. The accessibility of such starches thus, needs to be considered.

3.2. Starch and its classification

Chemically, starches are polysaccharides, consisting of a α -D-glucopyranosyl units bonded together with α -D-(1 \rightarrow 4) and/or α -D-(1 \rightarrow 6) linkages. The starch is composed of 2 principal structural components: amylose, the straight chain polyglucan consisting of approximately 1000, α -D-(1 \rightarrow 4) linked glucoses; and amylopectin, the branched glucans, consisting of approximately 4000 glucose units with branches occurring as α -D-(1 \rightarrow 6) linkages (Sharma, Yadav, & Ritika, 2008; Haralampu, 2000). X-ray diffraction studies on oriented amylase fibres identified the presence of two types of amylase: A and B (Galliard 1987).

These types depend in part on the chain length creating the amylopectin lattice, the density of grouping within the granules, and the presence of water (Wu and Sarko 1978). Even though type A and type B are real crystalline modifications, there is a third type C which is a mixed form of both A and B. The important features of the types of starches are as follows.

Type A. The type A structure has amylopectin of chain lengths of 23 to 29 glucose units. The hydrogen bonds between the hydroxyl groups of the chains of amylopectin molecules lead to the formation of outer double helical structure. Between these micelles, linear chains of amylose moieties are packed by forming hydrogen bonds with outer linear chains of amylopectin. This

pattern is very common in cereals. Type A is analogous to type B, except that the central channel is tied up by another double helix, making the grouping closer. In this form, only 8 molecules of water per unit cell are included between the double helices. Amylopectin (107 to 109 g/mol) is well branched and has an average DP of 2 million, making it one of the major molecules in nature. Chain lengths of 20 to 25 glucose units between branch points are characteristic. Its structure is frequently described by a cluster model which acquires more credit when Hizukuri hypothesized that amylopectin chains are either found within a single cluster or serve to attach 2 or more clusters (Hizukuri 1986; Thompson 2000). Short chains (A) of DP 12-16 that can form double helices are arranged in clusters.

Type B. The type B structure is made of amylopectin of chain lengths of 30 to 44 glucose molecules with water inter-diffuse. This is the usual model found in starches of raw potato, banana and amylose-rich starches. The structural entities of type B are double helices, which are grouped in an antiparallel, hexagonal mode. The central channel enclosed by 6 double helices is filled with water (36 H₂O/unit cell)

Type C. The type C structure consists of amylopectin of chain lengths of 26 to 29 glucose molecules, a mix of type A and type B, which is common in peas and beans. An additional form, called type V, is found in swollen granules.

Based on the action of enzymes

Based on the enzyme activity, starches can be also grouped according to their features when incubated with enzymes without previous exposure to dispersing agents Berry (1986). These are rapidly digestible starch (RDS) which are composed primarily of amorphous and dispersed starch, slowly digestible starch (SDS) which consists of physically inaccessible amorphous starch and raw starch and resistant starch.

Resistant starch is the sum of starch and starch products that resist digestion as they pass via the gastrointestinal tract. Resistant starch is not hydrolyzed to D-glucose in the small intestine within 120 min of being consumed, but it is fermented in the colon. Some studies demonstrated that RS is a linear molecule of α -1,4-D-glucan, basically originated from the retrograded amylose fraction, and has almost low molecular weight (1.2×10^5 Da) (Tharanathan, 2002). RS is an extremely wide and assorted range of components and they exist 4 subtypes (RS1–RS4). Currently, these are typically defined based on their physical and chemical attributes (Nugent, 2005). Table 5 summarizes the different subtypes of resistant starch, their resistance to digestion in the small intestine and food sources of every subtype of RS (Lunn & Buttriss, 2007).

Table 5 .Types of resistant starch, their resistance to digestion in small intestine and food sources. Sources: Bird et al. (2008), Sharma et al. (2008), Rajman et al. (2007), Lunn and Buttriss (2007), Sajilata et al. (2006), and Nugent, (2005).

Type of starch	Description	Digestion in small intestine	Resistance reduced by	Food sources
RS1	Physically inaccessible to digestion by entrapment in a non-digestible matrix	Slow rate; partial degree digested If properly milled	Milling, chewing	Whole or partly milled grains and seeds, legumes, pasta
RS2	Ungelatinized resistant granules with type B crystallinity, slowly hydrolyzed by amylase	Very slow rate; little degree digested when freshly cooked	Food processing and cooking	Raw potatoes, green bananas, some legumes, high-amylose starches
RS3	Retrograded starch formed when starch containing foods are cooked and cooled	Slow rate; partial degree Reversible digestion: digestibility improved by reheating	Processing conditions	Cooked and cooled potatoes, bread, corn flakes, food products with prolonged and/or repeated moist heat treatment
RS4	Selected chemically-modified resistant starches and industrially processed food ingredients	As a result of chemical modification, can resist hydrolysis	Less susceptible to digestibility in vitro	Some fibre: drinks, foods in which modified starches have been used (certain breads and cakes)

The natural types of RS are often degraded when processed. The production of resistant starch typically entails partial acid hydrolysis and hydrothermal treatments, heating, retrogradation, extrusion cooking, chemical modification and repolymerisation (Charalampopoulos et al., 2002). The four distinct subtypes of RS in foods are: (1) RS1 –is enclosed within whole or partially milled grains or seeds is the physically unavailable starch; (2) RS2 – found in some types of raw starch foods (such as banana and potato) and high-amylose (high-amylose corn) starches; (3) RS3 – retrograded starch (either processed from unmodified starch or resultant from food processing applications); (4) RS4 – starches that are chemically modified to be resistant to enzymatic digestion (such as some starch ethers, starch esters, and cross-linked starches) (Ratnayake & Jackson, 2008; Sanz, Salvador, Baixauli, & Fiszman, 2009). RS1 and RS2 are part of starch forms, which are gradually and partially digested in the small intestine. RS1 is the starch which is physically

unavailable to digestion, because of the presence of intact cell walls in grains, seeds or tubers (Hernández et al, 2008). RS1 is heat stable in the majority of normal cooking techniques, which facilitates its use as an ingredient in an ample variety of traditional foods (Sajilata, Singhal & Kulkarni, 2006). RS2 are native, uncooked starch food, such as raw potato or banana starches, whose crystallinity makes them weakly accessible to hydrolysis (Hernández et al., 2008). They are sheltered from digestion by the conformation or structure of the starch particle. This solid structure limits the availability of digestive enzymes, diverse amylases, and accounts for the resistant nature of RS2 such as, ungelatinized starch. A particular type of RS2 is distinctive as it keeps its structure and resistance during the processing and preparation of many foods; this RS2 is called high-amylose maize starch (Wepner et al., 1999).

RS3 are non-granular starch-derived materials that are not digested. RS3 types are usually produced through the cooling of gelatinized starch (Wepner et al., 1999). So they can be formed in cooked foods that are kept at low or room temperature (Hernández et al., 2008). Most moist-heated foods thus have some RS3 (Sajilata et al., 2006). RS3 is of mostly important, because of its thermal stability. This enables it to be stable in most normal cooking techniques, and permits its use as an ingredient in a wide variety of conventional foods (Haralampu, 2000). It is determined chemically as the fraction resistant to both dispersion by boiling and enzyme digestion. It can only be separated with KOH or dimethyl sulphoxide (Asp and Bjorck 1992). RS3 is completely resistant to digestion by pancreatic amylases. Food processing, which entails heat and moisture, usually break down RS1 and RS2 converting them into RS3 (Faraj et al., 2004). RS3 have a higher WHC than granular starch (Sanz et al., 2008a). Some examples of RS3 are cooked and chilled potatoes and corn-flakes (Wepner et al., 1999). About 80–90% of the glucose produced by the enzymatic hydrolysis of normal starch is metabolized in the human body. In general, the digestibility of RS depends on the type and source of RS consumed. Of the total amount of RS3 found in corn and wheat, about 84% and 65%, respectively, are degraded by bacterial fermentation in the colon. Likewise, 89% of RS2 from raw potato and 96% of RS2 from green banana are degraded by bacterial fermentation in the colon. The degradation of RS is also influenced by diverse food processing conditions under which RS is produced. The digestibility of RS was also found to be different per individual. This inconsistency can be endorsed to individual differences toward enzymatic responses (Sharma et al., 2008). The extended intake of an amylose-rich diet enhances fasting triglyceride and cholesterol levels more than an analogous amylopectin-rich diet (Mikulíková et al., 2008). Besides the three other types of RS, chemically-modified starch has been defined as RS type 4, analogous to resistant oligosaccharides and polydextrose (Wepner et al., 1999).

RS4 can be further classified into four subtypes according to their solubility in water and the analytical methods by which they can be analyzed (Nugent, 2005). They can be made by chemical modifications, such as conversion, substitution, or cross-linking, which may avoid its digestion by blocking the accessibility of enzyme and creating uncommon links such as $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ links (Kim et al., 2008; Sajilata et al., 2006).

Various forms of RS2 and RS3 of different origins and amount of RS are accessible as commercial ingredients in the European market to be incorporated in food. As RS4 is produced by chemically-modified starches, with a far higher number of modifications than the common chemically-modified starches approved in Europe, it is an innovative food not yet agreed by the European Union.

However, RS4 is approved in Japan (Sanz et al., 2008b; Lunn & Buttriss, 2007). In addition to the structural aspects described above whereby the chemical structure of starch can affect the percentage of RS present, other aspects intrinsic to starchy foods can influence α -amylase activity and thus starch breakdown. These are the production of amylose–lipid complexes, the presence of native α -amylase inhibitors and also non-starch polysaccharides, all of which can directly influence α -amylase activity. Extrinsic additives, like phosphorus, can also combine to starch, making it more or less disposed to degradation. Moreover, physiological factors can influence the amount of RS in a food. Increased chewing reduces particle size (smaller particles are easily digested in the gut), whereas intra-individual variations in transit time and biological factors (e.g. menstrual cycle) also influence the digestibility of starch.

3.3. Food sources of resistant starch

Factors that determine the resistance of starch to digestion comprise the physical form of grains or seeds in which starch is situated, mostly if these are whole or partially dislocated, size and type of starch granules, connections between starch and other dietary compounds, and cooking and food processing, especially cooking and cooling (Slavin, 2004). The digestibility of starch in rice and wheat is raised by milling to flour (Sajilata et al., 2006). As a food ingredient, RS has a low calorific (8 kJ/g) index in contrast to fully digestible starch (15 kJ/g); nevertheless, it can be added into a wide variety of food products such as baked products without affecting the processing features or the appearance and taste of the product (Rochfort & Panozzo, 2007).

Unripe banana is considered the RS-richest non-processed food. Some studies reported that consumption of unripe bananas confers health benefits to human, a fact often related to its high RS content, which varies between 47% and 57%. Even though banana constitutes an alternative form of indigestible carbohydrates, it is important to take into account that, when the unripe fruit is cooked,

its endogenous RS become digestible (Rodríguez et al., 2008). As a percentage of total starch, potato starch has the highest RS concentration and corn starch has the lowest. Raw potato starch holds 75% of RS as a percentage of total starch (TS). Amylomaize contains typically amylose, which has been reported to decrease not only digestibility but also blood insulin and glucose values in humans (Bednar et al., 2001).

RS content are low for the flour group as a whole. Cereal flours have an A-type crystalline pattern, which is more willingly degraded than raw cereals that are not as highly processed as flours. Consequently, cereal flours have more RDS and SDS than RS. The RS concentrations are fivefold higher in the cereal whole grains than in the flours (Bednar et al., 2001). Cooked grain products have modest levels of RS (mean 9.6% as a percentage of TS). Starch pasta is more slowly digested as the result of the heavily packed starch in the food (Bednar et al., 2001). Legumes are recognized for their high content of both soluble and insoluble dietary fibre. Pulse grains are high in RS and maintain their functionality even after cooking (Rochfort & Panozzo, 2007). Legume starches contain higher amylose levels than cereal and pseudocereal starches (Mikulíková et al., 2008). A possible cause for the higher RS concentrations in legumes might be the connection between starch and protein. When red kidney beans are preincubated with pepsin, there is a raise in their susceptibility to amylolytic enzyme (Bednar et al., 2001). Prepared legumes are quickly retrograded, thus decreasing the digestion. Processed legumes have significant content of RS3. The digestibility of legume starch is much lower than that of cereal starch. The higher amount of amylose in legumes, which possibly leads to a higher RS content, can be responsible of their low digestibility. It has been demonstrated that high-amylose cereal starch is slowly digested (Tharanathan & Mahadevamma, 2003). There is a huge difference in the content of resistant starch in seeds of leguminous plants (from 80% to only a few percent). However, the influence of processing is important for resistant starch content. Hydrothermal processing may result to a raise or reduction in the fraction of resistant starch (depending on the factors of processing and varieties of legumes) (Giczewska & Borowska, 2003).

3.4. Processing conditions of resistant starch

Gelatinization and retrogradation processes leading to the formation of RS can be influence by processing methods. They are of great importance for the food industry as they can be applied for the increase of RS in food products. Processes techniques such as baking, pasta production, extrusion cooking, autoclaving, have been reported to affect the content of RS in foods (Siljestrom and Asp 1985; Bjorck and Nyman 1987; Siljestrom and others 1989; Muir and O'Dea 1992; Rabe

and Sievert 1992). Low levels of RS from 1.5% to 8% on a dry basis have been found on cereal flours processing. Legumes are good sources of RS because of their stable crystalline structure (type C) whereas processing cereal decrease in RS content because of their type A crystal structure which is less stable (Ring et al., 1988). During the food preparation the crystalline structure of RS can break up resulting in decrease of RS content. Extrusion cooking may raise levels of RS (Haralampu 2000). Many studies have been done on the effect of processing on RS contents (Siljestrom and Bjorck 1990; Parchure and Kulkarni 1997; Kavita and others 1998).

RS amount is also influenced by steam cooking. For example 19% to 31%, DM basis of RS have been observed in steam-heated legumes (Tovar & Melito, 1996). In conventional and high-pressure steamed beans, RS was 3 to 5 times higher than raw pulses, probably due to retrogradation which causes indigestibility. Long steam cooking and short dry pressure heating are responsible for production of other types of non-digestible starch consequently of the decreased of total starch content of whole beans by 2% to 3% (DM basis) (Tovar and Melito 1996).

Baking increases RS content. Westerlund et al., (1989) evaluated the effect of baking on RS formation; white bread was baked and slicing into 3 fractions (crumb, inner crust, and outer crust). They observed that starch content was high in dough and low in outer crust after baking for 35 min. On the contrary, RS content was highest in crumb and lowest in dough in the same baking condition. Usually bread baked at low-temperature/long-time has higher amounts of RS than bread baked under normal conditions (Liljeberg et al., 1996). Adding lactic acid in bread making can increase RS while malt does not have effect on RS content. High-amylose barley flour has been reported to have high level of RS when long-time baked bread was applied.

In some foods, starch that is physically inaccessible is an important fraction of the total undigested starch in vivo. Schweizer et al., (1990) reported that 20% of starch not digested from a diet enclosing bean flour with intact cells. About half of the undigested starch was retrograded amylose. RS content is reduced by fermentation. A study done with sorghum flour cv. Tabat mixed with water and prefermented dough, and fermented at 37 °C for 36 h reported an enhance of starch digestibility and a reduction of RS amount and total starch (Abd-Elmoneim et al., 2004). The content of RS decrease in some fermented products, such as idlis and dhoklas (Kavita et al., 1998).

3.5. Beneficial physiological effects of resistant starch

RS is one of the most abundant dietary sources of non-digestible carbohydrates (Nugent, 2005) and might be as important as NSP in improving large bowel health and preventing bowel inflammatory diseases (IBD) and colorectal cancer (CRC) (Topping, Anthony, & Bird, 2003) but has a smaller

influence on lipid and glucose metabolism (Nugent, 2005). Numerous physiological effects have been attributed to RS, which have been demonstrated to be beneficial for health (Sajilata et al., 2006 and are listed in Table 6. The physiological properties of resistant starch (and so the potential health benefit) can differ widely depending on the study design and variations in the source, type and dose of resistant starch consumed (Buttriss & Stokes, 2008; Nugent, 2005). It is probable that modern processing and food consumption practices are responsible of the low consumption of RS, which might cause the rise in serious large bowel diseases in occidental countries. This can create opportunities for the formulation of new cereal products and starch-based ingredients for food products which can improve public health. These products may also be applied clinically (Topping et al., 2003). RS operates mainly through its large bowel bacterial fermentation products which are, in adults, short-chain fatty acids (SCFA) (Topping et al., 2003) but attention is increasing in its prebiotic properties.

Table 6 .Physiological effects of resistant starch. Sources: Grabitske and Slavin (2009), Sharma et al. (2008), Scholz-Ahrens et al. (2007), Brouns et al. (2002), and Nugent (2005).

Protective effect	Potential physiological effects
Diabetes	Control of glycaemic and insulinemic responses
Colorectal cancer, ulcerative colitis, inflammatory bowel disease, diverticulitis and constipation	Improved bowel health
Cardiovascular disease, lipid metabolism syndrome, cholesterol and triglycerides	Improved blood lipid profile
Colonic health	Prebiotic and culture protagonist
Obesity	Increased satiety and reduced energy intake
Osteoporosis, enhanced calcium absorption	Increased micronutrient absorption

There is also increasing attention in using RS to reduce the energy value and accessible carbohydrate content of foods. RS can also be applied to enhance the fibre content of foods and its potential to accelerate the beginning of satiation and to decrease the glycemic response is still studied. RS can improve gut health, and can be a vehicle to increase the total dietary fibre content of food, mainly those which have low energy rate and/or total carbohydrate content (Nugent, 2005).

3.5.1. Prevention of colonic cancer

It has been proved that butyrate may decrease the risk of malignant changes in cells. It has been reported that rats fed with RS preparations demonstrated an increase in fecal bulking and decrease of fecal pH, in addition to greater production of SCFA, which are related to the declined incidence of colon cancer, which have been hypothesized to be similar to soluble dietary fibre effects (Ferguson et al., 2000; Tharanathan & Mahadevamma, 2003).

DF and RS, as they are degraded in the large intestine, produce high levels of butyric acid or its salts (Sharma et al., 2008) as in vitro experiments with human faeces have revealed (Sajilata et al., 2006). In 2003, Champ et al. also established a specific role of resistant starch in the stimulation of ability to produce butyric acid. As butyrate is one of the major energy substrates for large intestinal epithelial cells and reduces the malignant transformation of such cells in vitro; this makes easily fermentable RS fractions especially interesting in preventing colonic cancer (Asp & Bjorck, 1992). Various studies observed that, butyrate can have an inhibitory effect on the growth and propagation of tumour cells in vitro by blocking one of the phase of cell cycle (G1) (Sharma et al., 2008). Bingham et al., (2003) observed that in individuals with a low to average intake of dietary fibre, by doubling dietary fibre consumption might reduce the risk of colorectal cancer by up to 40%. On the other hand, there was no correlation between dietary NSP and large bowel cancer (Sharma et al., 2008). Nevertheless, when RS was mixed with an insoluble dietary fibre, such as wheat bran, high production of SCFA was reported in the faeces, especially of butyrate (Leu, Hu, & Young, 2002). In 2008, Liu and Xu demonstrated that RS dose-dependently restrained the formation of colonic aberrant crypt foci (ACF) only when it was present during the promotion phase to a genotoxic carcinogen in the middle and distal colon, supposing that consumption of RS can delay growth and/or the development of neoplastic lesions in the colon. Thus, colon tumorigenesis can be highly susceptible to the type of diet. Adults with preneoplastic lesions in their colon can as a result gain from dietary RS. This suggests the efficacy of RS as a preventive agent for individuals at high risk for colon cancer development (Liu & Xu, 2008).

3.5.2. Hypoglycemic effects

The GI (glycemic index) of starchy foods can be associated to diverse factors such as the amylose/amylopectin ratio, the original environment of the starch granule, gelatinization of starch, water content and baking temperature of the processed foods. Consequently, the factors influencing the GI values are related to those of RS formation. With glucose as reference, standard GI values are about 10 for starch in legumes to 100 in some potato or rice products and breakfast cereals (Sharma et al., 2008), so foods having RS decrease the rate of digestion. The slow digestion of RS

implied its use in management of glucose release (Sajilata et al., 2006) and for that reason, a decrease in insulin response and better access to the use of stored fat can be predictable (Nugent, 2005). This is undoubtedly essential for diabetes and had contributed to most important changes in dietary recommendations for diabetics (Cummings, Edmond, & Magee, 2004). The metabolism of RS arises 5–7 h after consumption, in contrast to normal cooked starch, which is digested instantly. Digestion over a 5–7 h period decreases postprandial glycemia and insulinemia and can increase the time of satiety (Raben et al., 1994; Reader et al., 1997). Many studies have been done on the effects of different types and doses of RS on glucose and insulin responses but the data are ambiguous (Sharma et al., 2008). In a humans study, Reader et al. (1997) demonstrated that the ingestion of RS3 decreased serum glucose and insulin levels compared to other carbohydrates. The study also reported that food containing RS reduced postprandial blood glucose and could promote metabolic control in type II diabetes (non-insulin dependent). Elevated GI values have been observed in humans consuming potatoes and cornflakes – foods that have significant amounts of retrograded starch (Truwell, 1992) generally, positive effects were reported shortly (within the first 2–8 h) after heavy meal (Higgins, 2004). RS have to supply at least 14% of total starch intake in order to give any benefits to glycemic or insulinaemic responses (Behall & Hallfrisch, 2002; Brown et al., 2003; Higgins, 2004). A study reported that RS decreases levels of glucose dependent insulintropic polypeptide m-RNA along the jejunum and ileum in both normal and type 2 diabetes rats (Shimada et al., 2008). Chemically-modified starches (RS4) have also been shown to produce diverse glucose responses. The effect of two experimental meals having 1–2% acetylated potato starch and beta cyclodextrin enriched potato starch (2–3%), respectively, was observed in humans and only the latter reduced the glucose levels. This can be the result of more distal absorption of β -cyclodextrin in the intestine or to retarded gastric emptying (Roben et al., 1997). Since RS has a low glycemic response, it can be use as a food ingredient to foods which help reduce the global GI value of the food (mainly if it is substituting readily absorbed forms of carbohydrate). The interest on RS as food ingredient is increase in food industry especially in the production of breads and cakes or similar products which usually may have had higher GI values (Nugent, 2005).

3.5.3. Resistant starch as a prebiotic

Prebiotics are indigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or more bacteria (probiotics) in the gastrointestinal tract and thereby exerting a health-promoting effect (Scholz-Ahrens et al., 2007; Roberfroid, 2000). Usually prebiotics are inulin and oligofructose, both naturally found in some fruits and vegetables

(e.g. bananas, chicory, Jerusalem artichokes, onions, garlic and leeks, and wheat), and other resistant oligosaccharides like inulin-type fructans (Buttriss & Stokes, 2008). Many studies on pigs and humans reported a time-dependent change in fecal and large bowel SCFA profiles, hypothesizing the probable interaction of RS with the consumed bacteria (Topping et al., 2003). RS has also been reported for use in probiotic formulations to promote the growth of such beneficial microorganisms as bifidobacterium (Brown et al., 1996). As RS approximately completely passes through the small intestine, it may be exploit as a growth substrate for probiotic microorganisms (Sajilata et al., 2006).

3.5.4. Hypocholesterolemic effects

Some studies in rats showed that RS may affect lipid metabolism, it have been observed a reductions in a number of measures of lipid metabolism. These are total lipids, total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), triglycerides and triglyceride-rich lipoproteins (Nugent, 2005). Hypocholesterolemic effects of RS have been widely established. In rats, RS diets (25% raw potato) clearly increase the fecal size and the fecal pool of SCFA, as well as SCFA absorption and decreased plasma cholesterol and triglyceride levels. In addition, concentration of cholesterol decreases in all lipoprotein fractions, particularly the HDL1 and a decreased concentration of triglycerides in the triglyceride rich lipoprotein fraction was observed (Sajilata et al., 2006). The bean starches reduce the levels of serum total cholesterol and VLDL + IDL + LDL cholesterol, raise the fecal concentration of short-chain fatty acids (in particular the butyric acid concentration), and raise fecal neutral sterol excretion. There are evidences that consumption of RS can drop off the serum cholesterol level in rats fed a cholesterol-free diet (De-Deckere et al., 1993; Hashimoto et al., 2006).

3.5.5. Inhibition of fat accumulation

Numerous authors have studied the possible use of RS to modify fat oxidation (Nugent, 2005) and diverse studies (Nugent, 2005; Sharma et al., 2008) have studied its potential as satiety agent and also as ingredient of weight management (Mikušová, et al., 2009), even though the results are still unsatisfactory. It is suggested that consuming an enriched diet in RS can enhance the mobilization and use of fat food as a direct result of a reduction in insulin secretion (Tapsell, 2004). Studies in humans would specify that enriched diets in RS do not influence total energy expenditure, carbohydrate oxidation or fat oxidation (Ranganathan et al., 1994; Tagliabue et al., 1995; Howe et

al.,1996; Raben et al., 1997). In an additional study on human participants, RS enriched breads produced more satiety than white breads between 70 and 120 min after eating (De Roos et al., 1995). Anderson et al.,(2002) shown that high-RS meals produced less satiety than low-RS meals 1-h post intake. Higgins et al., (2004) evaluated the association between the RS content of a meal and postprandial fat oxidation, concluding that substituting 5.4% of total dietary carbohydrates with RS may considerably raise postprandial lipid oxidation and possibly diminish fat accumulation in the long term. Keenan et al. (2006) demonstrated that the incorporation of resistant starch in the diet as a bioactive functional food compound is a natural, endogenous approach to augment gut hormones that are efficient in reducing energy intake, therefore can be an useful natural method for the treatment of obesity.

4. FRUCTANS

4.1. Introduction

Fructans are carbohydrates constituted of monomers of fructose with various DP, some of these have a terminal unit of D-glucose. The fructans are classified according to the variations in their DP and the type of links between the various monomers, the largest group is the inulin-type fructosyl-fructose linkages with β (2 \rightarrow 1) (Roberfroid et al., 2010). The classification according to the DP can vary from 2 to 60 (IUPAC - IUB, 1982) distinguishing between those with DP <10 and those with DP > 10. The first are defined as fructo-oligosaccharides (or oligofructose FOS), among those the most frequent are: kestose, nystose and fructosyl-nystose, and the others are generally defined as inulin (Van Loo et al, 1995).

The fructans are found in some monocotyledonous plants such as wheat, rye, barley and bananas, *Liliaceae* such as onion, garlic and leek as well as *Compositae* and accompany inulin in roots of chicory (*Cichorium intybus* L.) and bulbs of Jerusalem artichoke (*Helianthus tuberosus*). In Gramineae, they are usually found in the early stages of seed maturation. Both inulin and fructo-oligosaccharides improve gut function and selectively stimulate the growth of bifidobacteria (Bouhnik et al 1999; Brighenti et al 1999; Kruse et al 1999).

Because of their β -configuration of the anomeric C2 in their fructose monomers, inulin, and oligofructose are resistant to hydrolysis by human digestive enzymes (α -glucosidase; maltase-isomaltase; sucrase) that are specific for glycosidic linkages. They have thus been classified as 'nondigestible' oligosaccharides (Englyst & Cummings, 1991; Schweizer et al, 1990, Roberfroid & Slavin, 2000). Two studies showed that 86-88 % of the ingested doses of fructans (10, 17 and 30 g) were recovered in the ileostomy effluent confirming that they are practically indigestible in the small intestine of man (Hessov & Knudsen, 1995; Ellegård et al, 1997). The percentage of recovery in the ileostomy effluent is similar to the pectin and slightly smaller than the cereal fibre (Englyst & Cummings, 1985) and potato (Schweizer et al., 1990). The decrease of fructans is attributable to fermentation by the endogenous microflora. Consequently, the selective stimulation of the intestinal microflora makes these molecules the prototype of prebiotics (Gibson et al., 1995; Bouhnik et al., 1996; Kleessen et al., 1997). Also the speed of fermentation is very important, in vitro studies have shown that molecules with DP > 10 such as inulin are fermented in a time frame of less than 50 % compared to molecules with degree of polymerization \leq 10 as oligofructose.

Studies have shown that large quantities of FOS are stored in the stems and grains of wheat for much of its growing cycle. In spring wheat kernels, FOS concentration was found to be almost 20% of the dry matter at 9 days after anthesis (DAA), and the content per kernel rapidly decreased thereafter (Escalada and Moss 1976). Schnyder *et al.* (1988) reported that FOS made up 27% of the kernel dry matter at anthesis. It increased from 0.3 mg per kernel at anthesis to a maximum of 1.2 mg per kernel at 7 DAA and decreased rapidly to 0.5 mg at 17 DAA. Therefore, it appears that maximum accumulation in wheat occurs between the 2nd and 3rd week after flowering, at the physiological stage termed the milky phase. The high levels of fructose polymers in immature wheat grains seem particularly encouraging and allow for the suggestion of harvesting wheat at the milky phase for the utilization of this material as a functional food (Mujoo & P.K.W. Ng., 2003)

4.2. Fermentation properties

Inulin and oligofructose are completely fermented by gut microbiota as they reach the colon intact. Studies involving consumption of inulin and oligofructose by humans reported that they are usually not detected in the stools (Lewis 1912; Alles *et al.* 1996; Castiglia-Delavaud *et al.* 1998). They are degraded into bacterial biomass, SCFAs, lactic acid and gases (CO₂ and H₂) by gut LAB. The fermentation product such as SCFAs (acetate, propionate and butyrate) is rapidly consumed by the microbiota or absorbed through the intestinal mucosa; only a very tiny portion is excreted in the stools. Butyrate is partly responsible of the maturation and preservation of the colonic epithelium and used by the mucosal cells. It has a role as putative preventive agent in carcinogenesis and ulcerative colitis and stimulates apoptosis of cancerous cells (Kleessen *et al.* 2001). Others SCFAs like mainly propionate and lactate can get to the general circulation, and be further used as energy source by the liver and the muscles. The caloric rate of inulin-type fructans is around 4.2 kJ (1 kcal)/gram and 6.3 kJ (1.5 kcal)/gram for inulin and oligofructose, respectively (Roberfroid *et al.* 1993).

4.3. Dietary fibre effects and bowel habits

Inulin and oligofructose are recognized and labelled as dietary fibre in almost all countries worldwide. They promote bowel functions and a relief of constipation throughout fermentation and stimulation of bacterial growth (Kleessen *et al.* 1997; Den Hond *et al.* 2000). These result as an increased stool weight, as bacteria contribute considerably to the faecal mass and water content of the stools. This cause stimulation of bowel peristalsis, facilitation excretion, and raise of stool

output and stool frequency (Gibson *et al.* 1995; Castiglia-Delavaud *et al.* 1998; Scholtens *et al.* 2006).

4.4. Prebiotic properties

Prebiotic is defined as ‘a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health’ (Gibson & Roberfroid 1995). Prebiotics are different of other dietary fibres because of their selective fermentation model. Prebiotic has been largely studied for their selective stimulation of the growth of colonic bifidobacteria, known as reference organisms of a well-balanced flora, in the complex microbial community that colonises the colon. Bifidobacteria have anti-pathogenic properties, as a result prevent or alleviate several gastrointestinal disorders. They might also modulate the immune system, influence allergic inflammation and play a key role in the establishment of a healthy balanced infant microbiota (Salminen *et al.* 2005). *In vitro* studies reported that inulin and oligofructose are usually fermented by bifidobacteria in anaerobic batch culture fermenters inoculated with either pure starters or mixed human faecal flora. In all conditions, the selective growth of some bifidobacteria strains was detected when oligofructose or inulin was incorporated in the medium as the only source of carbon and energy. Other tested carbohydrate substrates (polydextrose, pectin and starch) did not selectively stimulate several bacterial genera (Wang & Gibson 1993; Gibson & Wang 1994a, 1994b; Hopkins *et al.* 1998). The inulin-type fructans prebiotic properties are a result of the capacity of bifidobacteria to degraded and use them specifically. Bifidobacteria have a β -fructo-furanosidase activity that consent them to efficiently use inulin and oligofructose as growth substrates in a competitive milieu such as the colon. Several intervention studies on humans, had demonstrated this selective ability to stimulate bifidobacteria, all detected a selective and considerable increase in bifidobacteria after consumption of inulin-type fructans (Gibson *et al.* 1995; Kleessen *et al.* 1997; Menne *et al.* 2000; Rao 2001; Tuohy *et al.* 2001; Harmsen *et al.* 2002; Bouhnik *et al.* 2007). It has been demonstrated that the intake of 15 g/day of either inulin or oligofructose in the diet of healthy volunteers selectively stimulated the growth of bifidobacteria, which was associated to a decrease in the counts of other bacteria such as bacteroides, clostridia and fusobacteria (Gibson *et al.*, 1995). With low portion as 5 g/day, oligofructose intake stimulated bifidobacteria by almost 1 logarithmic unit after 11 days (Rao 2001). Similar findings were observed after ingestion of 5 g/day of inulin (Bouhnik *et al.* 2007). Molecular-based applications, such as fluorescence *in situ* hybridisation, also corroborated a significant increase in bifidobacteria counts as a result of inulin-type fructans ingestion (Tuohy *et*

al. 2001; Harmsen *et al.* 2002). Contrary to other prebiotic studies evaluating changes in bacterial counts in the colonic lumen (planktonic flora), Langlands *et al.* (2004) considered the impact of a combination of 7.5 g/day of oligofructose and 7.5 g/day of long-chain inulin, given for 2 weeks to volunteers previous to colonoscopy, on the mucosa-associated intestinal flora. Samples for the Biopsy were collected from the colonic mucosa, permitting the quantification of the impact of the prebiotic on the mucosa-associated flora. Prebiotic consumption significantly ($P \leq 0.05$) increased mucosal bifidobacteria and LAB in both the proximal and distal parts of the colon. The microbiota colonising the epithelial surface of the intestine probably have a specific role in modulating the immune system reactivity. Collecting more information in humans studies, allow Roberfroid (2005) to conclude that the daily dose of a prebiotic is not correlated to the level of the bifidogenic response. At the population level, the prebiotic value is correlated with the basal composition of the faecal flora and is inversely proportional to the number of bifidobacteria initially present in the colon. Participants with a low initial population levels will be subject to a higher bifidobacteria raise after inulin-type fructans intake. Therefore, inulin and oligofructose both have important bifidogenic activities, and based on available data, the quantitative increase in bifidobacteria counts appears to be moderately independent of the fructans chain length. Nevertheless, a variation in profile and rate of fermentation appeared among components with different chain length distribution. By comparing the degradation rate of inulin-type fructans during *in vitro* fermentations, Roberfroid *et al.* (1998) demonstrated that the rate of fermentation of fructans with longer chains (DP>10) was about twice as slow as that of molecules with a DP <10. Meaning that oligofructose is rapidly fermented in the proximal part of the colon. On the other hand, longer-chain molecules (as in HP-inulin), demonstrated a slower fermentation rate and reached more distal parts of the colon, which are primarily susceptible to colonic chronic disease. Oligofructose-enriched inulin (Synergy1) unites both short-chain oligofructose and long-chain inulin, resulted in a larger spread and more sustained fermentation pattern that will benefit a more extended length of the colon.

4.5. Enhanced host resistance and colonic protection

Scientific evidences demonstrated that gut microbiota inhibits the growth of pathogens. As the numbers of bifidobacteria increase, prebiotics reduce the proliferation of pathogens bacteria such as *Escherichia coli*, *Campylobacter jejuni*, *Salmonella enteritidis* or *Clostridium perfringens* (Wang & Gibson, 1993; Fooks & Gibson, 2002). The production of SCFAs resulting in lowering of the colonic pH helps to prevention the overgrowth of pH-sensitive pathogenic bacteria. Human

intervention studies reported that inulin and oligofructose have a positive influence on the gut-associated lymphoid tissue. Mutually, these effects endow to strengthening our resistance towards colonization and translocation of pathogens, accelerate the recovery of the gastrointestinal tract after intestinal disorders, and improve disease symptoms. Most feeding interventions on human evaluated the modulations of the microbiota in healthy volunteers, which consequently contribute to maintaining health and wellbeing, particularly through an increased barrier function and colonisation resistance in the gut. An important area on the study of prebiotics effects comprises volunteers that are already subjected to gastrointestinal disease and susceptible to infections. Lewis *et al.* (2005) observed that oligofructose increased the number of bifidobacterial and decreased relapses of diarrhoea in patients hospitalized for *Clostridium difficile*-associated diarrhoea. Inulin-type fructans influence the risk of chronic colonic disease, and reduce inflammation in ulcerative colitis (Furrie *et al.*, 2005) or disease activity in Crohn's disease (Lindsay *et al.* 2006) modifying the composition of the microbiota, have been shown additionally to their bifidogenic properties. Beyond their gastrointestinal activities inulin-type fructans also generate favourable metabolic conditions that positively modulate mineral absorption (mainly calcium), so improving host health. Furthermore, inulin and oligofructose may influence enteroendocrine functions and modulate hormones involved in the regulation of appetite and satiety.

4.6. Improved mineral bioavailability

Experimental studies on animal models reported that inulin-type fructans have the ability to increase calcium and magnesium absorption. This result was observed in young growing rats (Delzenne *et al.* 1995), and also in ovariectomised female rats (Scholz-Ahrens *et al.* 2002), an experimental model for postmenopausal osteoporosis. In this last model, bone mineralisation in femur and lumbar vertebrae was also improved and prevented ovariectomy-induced loss of trabecular bone (tibia) structure. These results imply that inulin-type fructans may have beneficial effects on bone loss generated by oestrogen deficiency. A positive effect on bone mineral density has been also validated in growing rats (Roberfroid *et al.* 2002). A study compared several inulin-type fructans (oligofructose, HP-inulin and Synergy1) and reported that all of them enhanced calcium absorption, but the highest increase was observed with an oligofructose-enriched inulin (Synergy1) combining both short and longer fructan chains (Coudray *et al.* 2003).

4.7. Technological advantages

The application of inulin and oligofructose as fibre ingredients usually improve food organoleptic properties. Because inulin is tasteless it does not negatively change the sensory properties of foods in which it is added. Long-chain inulins are used as fat substitute resulting from their aptitude to stabilise water into a particle gel network with a fat-like, creamy texture. They had the mouth feel similar to fat. They are also employed as foams and emulsions stabilizers. On the other hand, oligofructose is highly soluble and has a quite sweet taste (about 35% compared with sucrose), that permits for natural sugar substitute. Inulin-type fructans have been already added in several foods such as dairy products, baked and cereal products, desserts, processed meat products, infant food, drinks, and meal replacers, therefore improving the nutritional profile of processed foods (Alexiou & Franck., 2008)

4.8. Inulin-type fructans and lipid metabolism

Inulin-type fructans also modulate lipids' metabolism affecting triglyceridaemia and cholesterolaemia as well as the distribution of lipids between the different lipoproteins improving beneficial health pattern (Delzenne et al. 2002). Several animal studies reported that inulin-type fructans influence the metabolism of the lipids mainly by reducing triglyceridaemia both in the fasted and the postprandial state. Feeding studies on animals reported a reduction of triglyceridaemia by 36 (SEM 3.4)% (mean value of eight different studies) where 10% inulin or oligofructose were added as supplement, its reduced cholesterolaemia effect is less stable, being statistically significant in only part of the studies so far reported. These reductions can be due to a decrease in the amount of VLDL particles with identical composition in lipids and the same size. In human feeding trials, inulin appeared to be more efficient than oligofructose in decreasing triglyceridaemia whereas in animals (especially in rats) both products were similarly active.

4.9. Inulin-type fructans and the body's defence functions

Numerous are the defence functions of the body concerning different organs, different mechanisms and different potential aggressors as target. Therefore the body is well protected and in a healthy person these multiple defence functions must exert an efficient protection. Nevertheless genetic factors, ageing, stress, insufficient physical activity and an unbalanced diet are all factors that can probably deteriorate these functions and thus create conditions for major sensitivity to external aggressions, both chemical and biological. In addition to their positive effect on the risk of diseases associated to dysfunction of gastrointestinal defence functions, inulin-type fructans may possibly

contribute to reduce the risk of diseases related to dysfunction of systemic defence functions. This has been observed particularly on experimental models of systemic infection, chemically induced mammary carcinogenesis, growth and metastasis of implanted tumour and cancer therapy. Experimental studies, on supplementing diet with inulin-type fructans reported that they:

- decrease systemic infection (Buddington et al. 2002);
- negatively modulate chemically induced mammary carcinogenesis (Taper & Roberfroid, 1999);
- retard the growth of implanted tumour (Taper et al. 1997, 1998);
- decrease the incidence of metastasis in a model involving the intramuscular transplantation of viable TLT cells into young male C3H mice (Taper & Roberfroid, 2000);
- potentiate the effectiveness of cancer therapy (Taper & Roberfroid, 2002a, b).

available data at this moment are almost exclusively experimental and there is an urgent need to design human intervention studies to test the hypotheses that are already supported by sound experimental data.

5. SOURDOUGH TECHNOLOGY: KEY TOOL IN BREAD MAKING

5.1. Introduction

Bread making is most likely one of the oldest technologies known to world. Bread is consumed in great quantity in the world in different types and forms depending on cultural habits. Flat breads are the oldest, most diverse, and most popular product in the world. Bread products and their production practices vary widely around the world. The purpose of bread making is to give more attraction, palatability and digestibility to cereal foods. The primary quality attributes of leavened wheat breads are high volume, soft and elastic crumb structure, good shelf life, and microbiological safety of the product (Cauvain 2003; Chavan and Jana 2008). Nonetheless, fresh bread usually have short shelf life and during its preservation, a number of chemical and physical alterations occur known as staling. Consequently of these changes, bread quality deteriorates progressively as it loses its freshness and crispiness while crumb firmness and rigidity increase. The pleasant aroma disappears and flavour becomes sour (Chavan and Jana 2008).

The use of the sourdough process is one of the oldest biotechnological processes in cereal food production. Sourdough bread is made from a mixture of flour and water that is fermented with lactic acid bacteria (LAB), mostly hetero-fermentative strains which releases lactic acid, and acetic acid in the mixture and so causing a pleasant sour-tasting end product.

5.2. Role of Ingredients in Making Bread

Production of bread involves important ingredients such as flour, yeast, and water. Each ingredient used has its own impact both in conventional as well as in sourdough-based product.

Flour

Wheat-based products are main source of nutrients in various regions of the world (Fincher and Stone 1986; Hoskeney et al., 1988). Flour is the key ingredient in bread making since it regulates the specific attributes of bakery products. It consists of protein, starch and other carbohydrates, ash, fibers, lipids, water, and small amounts of vitamins, minerals, and enzymes. Wheat flour is the most frequent flour used. Milling greatly influences the protein content as it decreases from 14.2% at 100% extraction to 12.7% at 66% extraction of flour (Pedersen 1994). The 2 essential types of protein that wheat flour have are gliadin and glutenin. When in their combination, (known as gluten) water is added for preparation of dough, they form a cohesive and elastic network, which provides to wheat its functional properties (Giannou et al., 2003; Chavan and Jana 2008). Noncovalent bonds, such as hydrogen bonds, ionic bonds, and hydrophobic bonds, are essential for

the aggregation of gliadins and glutenins and involve structure and physical properties of dough. The quantity and quality of gluten proteins mostly determine dough rheological and textural properties (Gan et al., 1995). Gas retention properties establish loaf volume and crumb structure of the resulting bread.

Yeast

Saccharomyces cerevisiae is the most common yeast used in bread making. Yeast cells metabolize fermentable sugars (glucose, fructose, sucrose, and maltose) under anaerobic conditions releasing carbon dioxide (CO₂), which works as a leavening agent and enhances dough volume (Giannou and others 2003; Chavan and Jana 2008).

Water

Water is essential for the formation of dough and is responsible for its viscosity. It is used for the dissolution of salt and sugars and helps the diffusion of yeast cells. Water is required for starch and sucrose hydrolysis. It is important for starch gelatinization during baking and contributes to oven spring through vaporization (Giannou and others 2003; Chavan and Jana 2008).

Sugars

Sugars are usually employed by yeast during the early stages of fermentation. Later, sugars are liberated for gas production by the endogenous enzymes. Sugars also operate as antiplasticizers delaying pasting of native starch or as antistaling ingredients inhibiting starch recrystallization (Giannou and others 2003; Chavan and Jana 2008).

Salt

Sodium chloride is an essential ingredient with a functional role in the bread making. Salt reinforces the gluten, influences the action of yeast, and thus influences the loaf volume. A small quantity of salt in dough ameliorates flavour and action of amylases helping to preserve a supply of maltose as food for the yeast (Giannou and others 2003; Chavan and Jana 2008).

Lipids

Lipids may be used in bread making either in the form of fats or oils and are generally referred to as shortening. They are an optional ingredient in bread but can improve dough handling and crumb appearance and contribute to product flavor. Lipids also ameliorate the preservation of the quality, softness, and moistness and contribute to bread texture (Giannou and others 2003; Chavan and Jana 2008).

5.3. Sourdough Processing

Spontaneous “sour” dough fermentation is one of the oldest cereal mainly used to leaven the dough to produce a more gaseous dough and thus a more aerated bread. Years ago, beer yeast was used for dough leavening (Spicher & Stephan 1999; Kulp & Lorenz 2003). The sourdough fermentation is a traditional process for improving bread quality and producing diverse wheat and rye breads (Thiele et al., 2002). Nowadays, the sourdough is applied in the production of breads, cakes, and crackers (Ottogalli et al., 1996). The distinctive characteristic of sourdough is primarily due to its microflora, mostly LAB and yeasts. Due to its microbial life, such dough is metabolically active and can be reactivated. These microorganisms are responsible of acid production and leavening upon addition of flour and water. The mechanisms in sourdough are complex (Hammes & Ganzle, 1998). Various flour qualities and process parameters influences the metabolic activity of the sourdough microflora. During fermentation, biochemical modifications occur in the carbohydrate and protein components of the flour due to the action of microbial and endogenous enzymes (Spicher, 1983).

5.3.1. Properties of Sourdough

The use of sourdough in wheat breads has gained attractiveness as a way to improve the quality and flavour of wheat breads. A broad range of traditional products depends on the application of sourdough fermentation to yield baked goods with specific characteristics. Some examples include the well-known Italian products associated with Christmas, Panettone, which originated in Milan. San Francisco sourdough, French breads, and soda crackers are other examples of wheat products that depend on the process of souring. The sourdough fermentation influences dough rheology at 2 stages, in sourdough itself, and in bread dough-containing sourdough. In dough, fermentation reduces elasticity and viscosity, while the incorporation of sourdough to final bread dough leads in less elastic and softer dough. The level of rheological modifications taking place in these dough and its influences on bread quality can be controlled by adjusting fermentation time and the ash content of flour during the prefermentation process (Clarke et al., 2004). Many inherent properties of sourdough depend on the metabolic activities of LAB: lactic fermentation, proteolysis, and synthesis of volatile compounds, antimould, and antiropiness production is among the most important activities during sourdough fermentation (Hammes & Ganzle, 1998; Gobbetti et al., 1999). Moreover, endogenous factors in cereal products (carbohydrates, nitrogen sources, minerals, lipids, and free fatty acids, and enzyme activities) and process parameters (temperature, dough yield [DY], oxygen, fermentation time, and number of sourdough propagation steps) markedly influence the microflora of sourdough and the features of leavened baked goods (Hammes and Ganzle 1998).

Dough yield

Sourdough can vary in its consistency. The sourdough fermentation can be performed as firm dough or as a liquid suspension of flour in water. This proportion between flour and water is called the DY and is defined as:

Dough yield = (amount of flour + amount of water) \times 100/amount of flour

The DY value of a sourdough will significantly influence the flavor profile of the sourdough. The firmer the sourdough (lower DY value), the more acetic acid is produced and the less lactic acid. The acidification rate is also influenced by the DY of a sourdough. The higher the DY, the faster the acidification will occur, most probably due to the better diffusion of the produced organic acids into the environment (Spicher & Stephan 1999).

Temperature

Temperature is the utmost important factor, as it influences DY more than acidification rate and also has an influence on the microbial composition of the sourdough. If backslopping is used where a part of the previous sourdough is used to inoculate the next fermentation, temperature plays a critical role because part of the microflora can be lost over the different sourdough refreshments if it is not controlled (Spicher & Stephan, 1999). Optimum temperatures for the growth of Lactobacilli are 30 to 40°C depending on strain and for yeasts 25 to 27°C. In general, a higher temperature, a higher water content of sourdough, and the utilization of wholemeal flour enhance the production of acids in wheat sourdoughs (Brummer & Lorenz, 1991).

Starter cultures

A 3rd parameter is the microflora used for the fermentation. Two main families can be distinguished: the heterofermentative and the homofermentative LAB. The flavor can easily be influenced changing the fermentation temperature as explained above. A commercially available sourdough starter commonly consists of mixtures of different LAB groups to assure good acidification and aromatization.

Titrateable acidity and pH

The titrateable acidity and pH of the dough are important during sourdough fermentation. In the initial phase, both acidity and pH remain constant, whereas during the intermediate phase, titrateable acidity increases due to the presence of yeast. During the long-term fermentation phase, the yeast presence becomes negative and titrateable acidity, and pH of the dough depends mainly on the LAB introduced into the system. The yeasts present in sourdough are only slightly influenced by lactic acid, but much more affected by acetic acid (Schulz, 1972).

Substrate

The substrate, mainly flour, used for sourdough fermentation is another parameter that significantly influences the sourdough. Ash content is important to determine flour grade and extraction rate, since the ash content of the bran is about 20 times that of endosperm (Matz, 1996). The ratio of bran to endosperm is higher in small kernels (Posner, 2000). The bran fraction contains more minerals and micronutrients that are important for the growth of LAB. The ash also influences the buffering capacity of the sourdough system that makes possible to reach a higher total titratable activity. The falling number of the flour is an indicator for the enzymatic activity of the flour. The lower the value the more amylase activity is present in the flour. At that moment, more free sugars will be available for the microflora to grow (Spicher & Stephan 1999).

5.3.2. Classification of Sourdough

Sourdoughs, on the basis of the technology used, are categorized into 3 types: Type I in which a sourdough is restarted using a part of the previous fermentation. These are the traditional sourdoughs. Type II is an industrial type of sourdough using adapted strains to start fermentation. This sourdough can be liquid, so it is easily pumpable in an industrial bakery. Type III, sourdough, which can be dried, is often used by industrial bakeries since the quality is constant and there are no longer end-product variations due to the freshly produced sourdough. The Type III sourdoughs are the most convenient to introduce authentic bread taste into the nowadays high-tech bakery industry. In industry, a lot of Type III sourdoughs are available (Bocker et al., 1995). Different drying techniques are used as well as liquid pasteurization, to achieve microbial stability. Spray-drying and drum-drying are the most commonly used drying techniques in Type III sourdough production.

5.3.3. Sourdough Microbiota

Sourdoughs are very complex biological ecosystems because of the microbial composition and all interactive effects among the bread-making processes and ingredients (Gobbetti et al., 1999). Cereal fermentations, namely sourdoughs, are dominated by specifically adapted LAB occurring at numbers above 10^8 CFU/g, which may be in coexistence or possibly in symbiosis with typical yeasts whose numbers are orders of magnitude lower (Gobbetti et al., 1999; Vogel et al., 2002). The majority of species regularly isolated from sourdough or used as sourdough starter belong, with only few exceptions, to 1 of the 4 genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella*. The highest number of different species (>23 species) is found in the genus *Lactobacillus*. There exists no discernible emphasis on any of the fermentation types classically dividing *Lactobacilli* in obligate homofermentative, facultative heterofermentative, and obligate heterofermentative strains.

The majority of yeasts found in sourdoughs have been allotted to the species *Candida milleri*, *C. holmii*, *S. exiguus*, and *S. cerevisiae* (Hammes & Ganzle, 1998). Most of the yeast formulations often contain LAB, especially *Lactobacilli* rather than *Pediococcus*, *Lactococcus*, and *Leuconostoc spp.* (Jenson 1998), which contributes a little to the aroma production through acidification of the dough during the limited processing period (Rothe & Ruttloff, 1983).

Yeasts are often associated with LAB in sourdough and the yeasts/LAB ratio is generally 1:100 (Gobbetti et al., 1994; Ottogalli et al., 1996). The yeasts found in sourdoughs belong to more than 20 species (Rossi, 1996; Stolz 1999; Gullo et al., 2003). Typical yeasts associated with LAB in sourdoughs are *S. exiguus*, *C. humilis* (formerly described as *C. milleri*), and *Issatchenkia orientalis* (*C. krusei*) (Spicher & Schroder, 1978; Gobbetti et al., 1995a, 1995b; Succi et al., 2003). Other yeast species detected in sourdough ecosystem are *Pichia anomala* as *Hansenula anomala*, *Saturnispora saitoi* as *P. saitoi*, *Torulaspora delbrueckii*, *Debaryomyces hansenii*, and *P. membranifaciens* (Gobbetti et al., 1994; Foschino & Galli, 1997; Succi et al., 2003). The variability in the number and type of yeast species in dough are affected by many factors such as dough hydration, level and the type of cereal used, the leavening temperature, and the sourdough maintenance temperature (Gobbetti et al., 1994).

5.3.4. Volatile Metabolites in Sourdough

Taste and aroma are definitely the most important attributes determining the quality of bread or baked cereals in general. Sourdough fermentation has a well-established role in improving flavour and structure of rye and wheat breads (Brummer & Lorenz, 2003). In principle, the whole grain or fractions of cereal grain can be modified by sourdough fermentation to improve nutritional value or promote healthfulness of cereal foods. Wholemeal flour is rich in fibre, minerals, vitamins, and many phytochemicals such as phenolic compounds, sterols, tocopherols, and tocotrienols, lignans, and phytic acid. With sourdough processes, the mouthfeel and palatability of wholemeal bread can be improved without removing any nutritionally important components (Salmenkallio-Marttila et al., 2001). Sourdough bread has a higher content of volatiles and, also, achieves higher scores in sensory tests compared to, for example, bread chemically acidified with lactic and acetic acid (Hansen et al., 1989a; Hansen & Hansen 1996). Some of the compounds present in bread have been shown to be related to the concentrations in the corresponding sourdoughs. As an example, the contents of methylpropanol (isobutanol), 2- and 3-methylbutanol (iso-pentanol), ethyl acetate, and ethyl lactate in 3 bakery sourdoughs were clearly related to the amounts of these compounds in the sourdoughs (Hansen & Lund, 1987; Lund et al., 1987).

There are 2 classes of flavour compounds produced during sourdough fermentation. Non volatile compounds, including organic acids, produced by homo- (Gobbetti et al., 1995a) and heterofermentative bacteria (Gobbetti et al., 1995b) that acidify, decrease pH, and contribute aroma to the bread dough (Galal et al., 1978; Barber et al., 1985). The 2nd class is volatile compounds that include alcohols, aldehydes, ketones, esters, and sulphur. All these compounds are produced by biological and biochemical actions during fermentation (Spicher 1983). The production of sufficient amounts of volatile compounds during fermentation needs a multiple-step process of about 12 to 24 h, while fermentation by baker's yeast alone is finished within a few hours. The generation of volatiles in sourdoughs is clearly influenced by the activity of the LAB and the sourdough yeasts. Factors influencing their activity, such as temperature and water content, will consequently influence the amounts of the metabolites formed. Generally, LAB are mostly responsible for acidification. The key degradation reaction of amino acids during dough fermentation is the Ehrlich pathway leading to aldehydes or the corresponding alcohols, respectively (Schieberle, 1996). Contrary, during baking, the Strecker reaction, initiated by α -dicarbonyl compounds such as methylglyoxal (2-oxopropanal), also leads to the respective aldehydes, but also to the corresponding acids (Hofmann & Schieberle, 2000). Because the chemical structures of the aldehydes generated during the Ehrlich pathway and the Strecker reaction are identical, it is a challenge to quantitatively differentiate between the amounts generated from the respective pathways. During dough fermentation, also phenylpropanoic acids, such as ferulic acid, were shown to be significantly increased (Hansen et al., 2002).

5.3.5. Beneficial Applications of Sourdough Technology

Dietary fibre (DF) in sourdough

There is growing consumer interest in health aspects of food, including functional food products with specific physiological functions of health relevance. However, good sensory properties remain a prerequisite for any successful food, and consumers also expect food to fulfil other criteria such as safety and convenience. The levels and also bioavailability of carbohydrates and various bioactive compounds can remarkably be influenced by processing. Another example of the potential of sourdough is the ability to modify the bran fraction of the grain (rich in fibre) in such a way that larger amounts of bran can be utilized in breads. The nutritional importance of DF has been demonstrated in many studies. A typical Western diet contains less than 20 g/d, whereas the recommended daily intake is 25 to 30 g. Currently, most people eat too little fibre and these low levels of DF in Western diet contribute to a long list of diseases, ranging in severity from dental

caries through constipation to obesity, colorectal cancer, coronary heart disease, and type 2 diabetes. The most common source of DF in baking is cereal bran, especially wheat bran. The use of barley bran derived from huskless or dehusked barley or oat bran derived from husked oat kernels is also becoming more widespread due to their high soluble DF content, in particular their content of mixed-linked β -glucans. According to Seibel (1983), addition of fibre causes the following technological changes: (1) increases DH, (2) results in a moister and shorter dough, (3) decreases fermentation tolerance (that is, dough is able to keep the optimum volume a shorter time during proofing), (4) decreases bread volume, (5) creates a crumb that is tense and nonelastic, and (6) creates flavour changes depending on type of fibre and bread type. However, additions of cereal bran, especially in such amounts that health benefits can be expected, cause severe problems in bread quality.

Flavor development

The flavour of leavened baked goods is influenced by raw materials, by sourdough fermentation, by the type of starters, and by proofing and baking conditions. The ratio between lactic acid and acetic acid is an important factor affecting the aroma of the final bread (Corsetti & Settanni, 2007), and it is influenced by the fermenting microorganism, the fermentation temperature, and the type of flour (Hansen & Schieberle, 2005). The use of sourdough can improve the taste and flavour of the bread (Rehman et al., 2006). The flavour of sourdough wheat bread is richer and more aromatic than wheat bread, a factor that can be attributed to the long fermentation time of sourdough (Brummer & Lorenz, 1991).

6. REFERENCES

- AACC International (1999). Definition of whole grain.
<http://www.aaccnet.org/definitions/wholegrain.asp> (accessed January 2010).
- AACC International (2006). AACC International comments on Part III of the Draft Guidance on Whole Grain Label Statements .
- Abd-Elmoneim-O-Elkhalifa, Schiffler B, Bernhard R. (2004). Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour. *Nahrung/Food* 48(2):91–4.
- Abdul-Hamid, A., & Luan, Y. S. (2000). Functional properties of dietary fiber prepared from defatted rice bran. *Food Chemistry*, 68, 15–19.
- Adam, A. et al.(2002). The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver of rats, *J. Nutr.*, 132, 1962.
- Adams JF & Engstrom A (2000). Helping consumers achieve recommended intakes of whole grain foods. *J Am Coll Nutr* 19, 339S–344S .
- Adil Gani, Wani SM, Masoodi FA and Gousia Hameed.(2012). Whole-Grain Cereal Bioactive Compounds and Their Health Benefits: A Review. *J Food Process Technol* 3:3
- Albertson AM & Tobelmann RC (1995). Consumption of grain and whole-grain foods by an American population during the years 1990 to 1992. *JAmDiet Assoc* 95, 703–704 .
- Alles MS, Hautvast JGA & Nagengast FM (1996). Fate of fructooligosaccharides in the human intestine. *British Journal of Nutrition* 76: 211–21.
- Aman, P. and Graham, H.(1987). Analysis of total and insoluble mixed-linked (1→3),(1→4)-β-D-glucans in barley and oats, *J. Agric. Food Chem.*, 35, 704.
- American Association of Cereal Chemists (2001). The definition of dietary fiber. *Cereal FoodsWorld* 46, 112–129 .
- Anderson, G. H., Catherine, N. L., & Woodend, D. M. (2002). Inverse association between the effect of carbohydrates on blood glucose and subsequent short term food intake in young men. *American Journal of Clinical Nutrition*, 76, 1023–1030.
- Andersson, A. A. M., Elfverson, C., Andersson, R., Regner, S., & Åman, P. (1999). Chemical and physical characteristics of different barley samples. *Journal of the Science of Food and Agriculture*, 79(7), 979–986.
- Andersson, A.A.M. et al.(1999). Chemical and physical characteristics of different barley samples, *J. Sci. Food Agric.*, 79, 979.
- Andersson, A.A.M., Andersson, R., and Aman, P.(2000). Air classification of barley flours, *Cereal*

- Chem., 77, 463.
- Andersson, R. and Åman, P. (2001). Cereal arabinoxylan: Occurrence, structure, and properties. *Adv. Dietary Fibre Technol.* 301-314.
- Andrewartha, K.A., Phillips, D.R. and Stone, B.A. (1979). Solution properties of wheat flour arabinoxylans and enzymatically modified arabinoxylans. *Carbohydr. Res.* 77, 191-204
- Anthony Fardet (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutrition Research Reviews* (2010), 23, 65–134
- AOAC International. (1995). Total, soluble and insoluble dietary fiber in foods. AOAC official method 991.43. *Official Methods of Analysis*, 16th ed .
- Asp NG. (1994). Nutritional classification of food carbohydrates. *Am J Clin Nutr* 59:S679–81.
- Asp, N. G. (1978). Critical evaluation of some suggested methods for assay of dietary fibre. *Journal Plant of Technology*, 3, 21–25.
- Asp, N. G., & Bjorck, I. (1992). Resistant starch. *Trends in Food Science & Technology*, 3(5), 111–114.
- Aspinall, G.O. (1959). Structural chemistry of the hemicelluloses. *Adv. Carbohydr. Chem.* 14, 429-468.
- Aspinall, G.O. (1970). Arabinans and Xylans. In: *Polysaccharides*. Pergamon Elmsford, New York, pp. 103-115.
- Aspinall, G.O. (1970). Arabinans and Xylans. In: *Polysaccharides*. Pergamon Elmsford, New York, pp. 103-115.
- Association of Official Analytical Chemists (2000). Method 991.43 for soluble and insoluble dietary fibre.
- Aura, A.-M. et al.(2005). Processing of rye bran influences both the fermentation of dietary fibre and the bioconversion of lignans by human faecal flora in vitro, *J. Sci. Food Agric.*, 85, 2085
- Bacic, A. and Stone, B. (1980). A (13)- and (14)-linked -glucan in the endosperm cell-walls of wheat. *Carbohydr. Res.* 82, 372-377.
- Bacic, A. and Stone, B.A. (1981). Chemistry and organization of aleurone cell wall components from wheat and barley. *Aust. J. Plant Physiol.* 8, 475-495.
- Balance, G.M. and Manners, D.J.(1978). Structural analysis: an enzymatic solubilization of barley endosperm cell walls, *Carbohydr. Res.*, 61, 107
- Bamforth, C.W. and Kanauchi, M.K.(2001). A simple model for the cell wall of the starchy endosperm in barley, *J. Inst. Brew.*, 107, 235.
- Barber S, Benedito de Barber C, Martinez-Anaya MA, Martinez J, Alberola J. (1985). Cambios en

- los ácidos orgánicos volátiles C2-C5 durante la fermentación de masas panarias preparados con masas madres comerciales y con cultivos puros de microorganismos. *Rev Agroquim Technol Aliment* 25:223–32.
- Bednar, G. E., Patil, A. R., Murray, S. M., Grieshop, C. M., Merchen, N. R., & Fahey, G. C. (2001). Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. *The Journal of Nutrition*, 131, 276–286.
- Behall, K. M., & Hallfrisch, J. (2002). Plasma glucose and insulin reduction after consumption of breads varying in amylose content. *European Journal of Clinical Nutrition*, 56, 913–920.
- Benedetti S, Primiterra M, Tagliamonte MC, Carnevali A, Gianotti A, Bordoni A, Canestari F (2012) Counteraction of oxidative damage in the rat liver by an ancient grain. *Nutrition* 28(4):436–441
- Berglund, P.T., Fastnaught, C.E., and Holm, E.T.(1994). Physicochemical and sensory evaluation of extruded high-fiber barley cereals, *Cereal Chem.*, 71, 91.
- Berry CS. (1986). Resistant starch. formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fiber. *J Cereal Sci* 4: 301–14.
- Bertin, C., Rouau, X., & Thibault, J.-F. (1988). Structure and properties of sugar beet fibres. *Journal of the Science of Food and Agriculture*, 44, 15–29.
- Bertin, C., Rouau, X., & Thibault, J.-F. (1988). Structure and properties of sugar beet fibres. *Journal of the Science of Food and Agriculture*, 44, 15–29.
- Betancur AD, Chel GL. (1997). Acid hydrolysis and characterization of *Canavalia ensiformis* starch. *J Agric Food Chem* 45:4237–41.
- Bhatty, R.S.(1986). Physicochemical and functional (breadmaking) properties of hull-less barley fractions, *Cereal Chem.* 63, 31.
- Bhatty, R.S.(1995). Laboratory and pilot plant extraction and purification of β -glucans from hull-less barley and oat brans, *J. Cereal Sci.*, 22, 163.
- Bhatty, R.S.(2003). The potential of hull-less barley, *Cereal Chem.*, 76, 589, 1999. Delaney, B. et al., β -Glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic Syrian golden hamsters, *J. Nutr.*, 133, 468 .
- Bhatty, R.S., MacGregor, A.W., and Rossnagel, B.G.(1991). Total and acid-soluble β -glucan content of hullless barley and its relationship to acid-extract viscosity, *Cereal Chem.*, 68, 221
- Biliaderis, C.G., Izydorczyk, M.S., and Rattan, O.(1995). Effect of arabinoxylans on breadmaking quality of wheat flours, *Food Chem.*, 5, 165.

- Bingham, S. A., Day, N. E., & Luben, R. (2003). Dietary fibre in food and protection against colorectal cancer in the European prospective investigation into cancer and nutrition (EPIC): An observational study. *The Lancet*, 361, 1496–1501
- Bjorck IM, Nyman ME. (1987). In vitro effects of phytic acid and polyphenols on starch digestion and fiber degradation. *J Food Sci* 52:1588–94.
- Bocker G, Stolz P, Hammes WP. (1995). Neue Erkenntnisse zum Ökosystem Sauerteig und zur Physiologie des Sauerteig-Typischen Stämme *Lactobacillus sanfrancisco* und *Lactobacillus pontis*. *Getreide Mehl und Brot* 49:370–74.
- Bouhnik Y, Raskine L, Champion K et al. (2007). Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans. *Nutrition Research* 27: 187–93.
- Bouhnik, Y., Vahedi, K., Achour, L., Attar, A., Salfati, J., Pochart, P., Marteau, P., Flourie, B., Bornet, F., and Rambaud, J. C. (1999). Shortchain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *J. Nutr.* 129:113-116.
- Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. (1995). A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* ; 274:1049–57.
- Braaten, J.T. et al.(1991). Oat gum lowers glucose and insulin after an oral glucose dose, *Am. J. Clin. Nutr.*, 53, 1425
- Braaten, J.T. et al.(1994). Oat β -glucan reduces blood cholesterol concentration in hypercholesterolemic subjects, *Eur. J. Clin. Nutr.*, 48, 465.
- Brighenti, F., Casiraghi, M. C., Canzi, E., and Ferrari, A. (1999) Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. *Eur. J. Clin. Nutr.* 53:726-733.
- Brown, I. L., McNaught, K. J., Ganly, R. N., Conway, P. L., Evans, A. J., Topping, D. L. et al. (1996). Probiotic compositions. Intl. Patent WO 96/08261/A1. Issued March 21, 1996. Univ. New South Wales, Burns Philip and Co., Limited, Burns Philip Res. and Dev. Pty., Mauri Lab. Pty. Ltd., Commw. Sci. Ind. Res. Org., Arnotts Biscuits Ltd., Goodman Fielder Ingredients Li, Goodman Fielder Ltd.
- Brown, M. A., Storlien, L. H., & Brown, I. L. (2003). Cooking attenuates the ability of high amylose meals to reduce plasma insulin concentrations in rats. *British Journal of Nutrition*, 90, 823–827.
- Brummer JM, Lorenz K. (2003). Preferments and sourdoughs in German breads. In: Kulp K, Lorenz K, editors. *Handbook of dough fermentations*. New York: Marcel Dekker Inc. p 247–67.
- Brümmer, J.M. & Lorentz, K. (1991). European developments in wheat sourdoughs. *Cereal Foods*

World 36: 310-314.

- Buddington KK, Donahoo JB & Buddington RK (2002). Dietary oligofructose and inulin protect mice from enteric and systemic pathogens and tumor inducers. *J Nutr* 132, 472–477.
- Buttriss, J. L., & Stokes, C. S. (2008). Dietary fibre and health: An overview. *British Nutrition Foundation, Nutrition Bulletin*, 33, 186–200.
- Caprez, A., Arrigoni, E., Amado, R., & Zeukom, H. (1986). Influence of different types of thermal treatment on the chemical composition and physical properties of wheat bran. *Journal of Cereal Science*, 4, 233–239.
- Carpita, N.C.(1996). Structure and biogenesis of the cell wall of grasses, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 47, 445.
- Castiglia-Delavaud C, Verdier E, Besle JM et al. (1998). Net energy value of non-starch polysaccharide isolates (sugarbeet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects. *British Journal of Nutrition* 80: 343–52.
- Cauvain S. (2003). Bread making: an overview. In *bread making improving quality*. Woodhead Publication Ltd. p 14.
- Cavallero, A., Empilli, S., Brighenti, F., and Stanca, A.M.(2002). High (1→3),(1→4) β-D glucan barley fractions in bread making and their effects on human glycemic response, *J. Cereal Sci.*, 36, 59.
- Champ, M., Langkilde, A. M., & Brovns, F. (2003). Advances in dietary fiber characterization 1. Definition of dietary fiber, physiological relevance, health benefits and analytical benefits. *Nutrition Research Reviews*, 16, 71–82.
- Charalampopoulos, D., Wang, R., Pandiella, S. S., & Webb, C. (2002). Application of cereals and cereal components in functional foods: A review. *International Journal of Food Microbiology*, 79, 131–141.
- Charles S. Brennan, Louise J. Cleary.(2005). The potential use of cereal (1/3,1/4)-β-D-glucans as functional food ingredients. *Journal of Cereal Science* 42, 1–13
- Chavan RS, Jana A. (2008). Frozen dough for bread making – a review. *Intern J Food Sci, Technol* Nut 2:9–27.
- Clarke CI, Schober TJ, Dockery P, O Sullican K, Arendt EK. (2004). Wheat sourdough fermentation: effects of time and acidification on fundamental rheological properties. *Cereal Chem* 81:409–17.
- Cleveland LE, Moshfegh AJ, Albertson AM, et al. (2000). Dietary intake of whole grains. *J Am Coll Nutr* 19, 331S–338S .

- Colleoni-Sirghie, M. et al.(2004). Prediction of β -glucan concentration based on viscosity evaluations of raw oat flours from high β -glucan and traditional oat lines, *Cereal Chem.*, 81, 434.
- Corsetti A, Settanni L. (2007). Lactobacilli in sourdough fermentation. *Food Res Intern* 40:539–58.
- Coudray C, Tressol JC, Gueux E et al. (2003). Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. *European Journal of Nutrition* 42: 91–8.
- Courtin, C. and Delcour, J.A.(2002). Arabinoxylans and endoxylanases in wheat flour breadmaking, *J. Cereal Sci.*, 35, 225.
- Courtin, C.M. and Delcour, J.A. (2001). Relative activity of endoxylanases towards waterextractable and water-unextractable arabinoxylan. *J. Cereal Sci.* 33, 301-312.
- Craig, S. A. S., Holden, J. F., & Khaled, M. Y. (2000). Determination of polydextrose as dietary fiber in foods. *Journal of AOAC International*, 83, 1006–1012.
- Crittenden, R. et al.(2002). In vitro fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria, *J. Sci. Food Agric.*, 82, 781.
- Cui, W., Wood, P.J., Blackwell, B., and Nikiforuk, J.(2000). Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β -Dglucan: comparison with other cereal β -D-glucans, *Carbohydr. Polym.*, 41, 249.
- Cummings JH, Englyst HN. (1991). Measurement of starch fermentation in the human large intestine. *Can J Physiol Pharmacol* 69:121–9.
- Cummings, J. H. and Englyst, H. N.(1991)of starch fermentation in the human large intestine, *Can. J. Physiol. Pharmacol*; 69: 121–129. Measurement
- Cummings, J. H., Edmond, L. M., & Magee, E. A. (2004). Dietary carbohydrates and health: Do we still need the fibre concept? *Clinical Nutrition supplements*, 1, 5–17.
- Dais , P. and Perlin, A.S., (1982). High-field, C-N.M.R. spectroscopy of β -D-glucans, amylopectin, and glycogen, *Carbohydr. Res.*, 100, 103
- Davidson, M. H., & McDonald, A. (1998). Fiber: Forms and function. *Nutrition Research*, 18, 617–624.
- De Roos, N., Heijnen, M. L., & De Graff, C. (1995). Resistant starch has little effect on appetite, food intake and insulin secretion of healthy young men. *European Journal of Clinical Nutrition*, 49, 532–541.
- De-Deckere, E. A. M., Kloots, W. J., & Van-Amelsvoort, J. M. M. (1993). Resistant starch decreases serum total cholesterol and triacylglycerol concentrations in rats. *Journal of Nutrition*, 123, 2142–2151.

- Delcour, J.A., Vanhamel, S., and Hoskeney, R.C.(1991). Physicochemical and functional properties of rye nonstarch polysaccharides. II. Impact of a fraction containing watersoluble pentosans and proteins on gluten-starch loaf volume, *Cereal Chem.*, 68, 72.
- Delzenne N, Aertssens J, Verplaetse N et al., (1995). Effect of fermentable fructo-oligosaccharides on energy and nutrients absorption in the rat. *Life Science* 57: 1579–87.
- Delzenne NM, Daubioul C, Neyrinck M, Lasa M & Taper HS (2002). Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. *Br J Nutr* 87,Suppl. 2, S255–S259.
- Den Hond E, Geypens B & Ghos Y (2000). Effect of high performance chicory inulin on constipation. *Nutrition Research* 20: 731–6.
- Ebringerová, A. and Heinze, T. (2000). Xylan and xylan derivatives - biopolymers with valuable properties. 1. Naturally occurring xylans structures, isolation procedures and properties. *Macromol. Rapid Commun.* 21, 542-556.
- Edwards, S., Chaplin, M.F., Blackwood, A.D., and Dettmar, P.W.(2003). Primary structure of arabinoxylans of ispaghula husk and wheat bran, *Proc. Nutr. Soc.*, 62, 217
- Eerlingen RC, Delcour JA. (1995). Formation, analysis, structure and properties of type III enzyme resistant starch. *J Cereal Sci* 21:1–8.
- Elleuch, M., Besbes, S., Roiseux, O., Blecker, C., Deroanne, C., Drira, N.-E., et al. (2008). Date flesh: Chemical composition and characteristics of the dietary fibre. *Food Chemistry*, 111, 676–682.
- Englyst HN, Kingman SM, Cummings JH. (1992). Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr* 46:S33–S50.
- Englyst, H. N., Quigley, M. E., & Hudson, G. J. (1994). Determination of dietary fiber as non-starch polysaccharides with gas–liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst*, 119, 1497–1509.
- Englyst, H. N., Quigley, M. E., Hudson, G. J., & Cummings, J. H. (1992). Determination of dietary fibre as non-starch polysaccharides by gas–liquid chromatography. *Analyst*, 117, 1707–1714.
- Erbersdobler FH. (1989). Factors affecting uptake and utilization of macro nutrients. In: Southgate DAT, Johnson IT, Fenwick GR, editors. *Nutrient availability: chemical and biological aspects*. Cambridge, U.K.: Royal Society of Chemistry. p 330–9.
- Escalada JA, Moss DN. (1976). Changes in nonstructural carbohydrate fractions of developing spring wheat kernels. *Crop Sci* 16:627–31.
- European Food Information Council (EUFIC) (2009). Whole grain fact sheet.

<http://www.eufic.org/article/en/page/BARCHIVE/expid/Whole-grain-Fact-Sheet/>(accessed January 2010).

- Faraj, A., Vasanathan, T., & Hoover, R. (2004). The effect of extrusion cooking on resistant starch formation in waxy and regular barley flours. *Food Research International*, 37, 517–525.
- Fastnaught, C.E., Berglund, P.T., Holm, E.T., and Fox, G.T.(1996). Genetic and environmental variation in beta-glucan content and quality parameters of barley for food, *Crop Sci.*, 36, 941
- Fastnaught, C.E., Berglund, P.T., Holm, E.T., and Fox, G.T.(1996). Genetic and environmental variation in beta-glucan content and quality parameters of barley for food, *Crop Sci.*, 36, 941.
- Faulds, C.B. et al.(2004) Arabinoxylan and mono- and dimeric ferulic acid release from brewer's grain and wheat bran by feruloyl esterases and glycosyl hydrolases from *Humicola insolens*, *Appl. Microbiol. Biotechnol.*, 64, 644.
- FDA. (2008).Health claims: fruits, vegetables, and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease. In *Code of Federal Regulations; Food and Drug Administration: Silver Spring, MD, USA Volume 2* .
- FDA.(2008). Health claims: Fiber-containing grain products, fruits and vegetables and cancer. In *Code of Federal Regulations; Food and Drug Administration: Silver Spring, MD, USA, Volume 2*
- Ferguson, L. R., Tasman-Jones, C., Englyst, H., & Harris, P. J. (2000). Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. *Nutrition & Cancer*, 36, 230–237.
- Fincher GB, Stone BA. (1986). Cell wall and their components in cereal grain technology. In: Pomeranz Y, editor. *Advances in cereal science and technology*. St. Paul, Minn.: American Association Cereal Chemists. p 207–95.
- Fincher, G.B. (1975). Morphology and chemical composition of barley endosperm cell walls. *J. Inst. Brewing* 81, 116-122.
- Fincher, G.B.(1975). Morphology and chemical composition of barley endosperm cell walls, *J. Inst. Brew.*, 81, 116
- Fischer, M.H. et al.(2004). The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk), *Carbohydr. Res.*, 339, 2009.
- Fleury, N., & Lahaye, M. (1991). Chemical and physico-chemical characterisation of fibres from *Lamaria digitata* (Kombu Breton): A physiological approach. *Journal of the Science of Food and Agriculture*, 55, 389–400.
- Food and Drug Administration (2006). *Draft Guidance for Industry and FDA Staff: Whole Grain*

Label Statements.

- Fooks LJ & Gibson GR (2002). In vitro investigation of the effect of probiotics and prebiotics on selected human intestinal pathogens. *FEMS Microbiology Ecology* 39: 67–75.
- Foschino R, Galli A. (1997). Italian style of life: pane, amore, lievito naturale. *Technol Aliment* 1:42–59.
- Fulcher, R.G. and Duke, T.K.R. (2002). Whole-grain structure and organization: Implications for nutritionists and processors. In: Whole-grain foods in health and disease. Marquart, L., Slavin, J.L. and Fulcher, R.G. (Eds.), American Association of Cereal Chemists, St. Paul, Minnesota, USA, pp. 9-45.
- Furrie E, MacFarlane S, Kennedy A et al. (2005). Synbiotic therapy (Bifidobacterium longum/Synergy1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 54: 242–9.
- Galal AM, Johnson JA, Varriano-Marston E. 1978. Lactic acid and volatile (C2-C5) organic acids of San Francisco sourdough French bread. *Cereal Chem* 55:461–68.
- Galliard T. (1987). In starch: properties and potential. Chichester, U.K.: John Wiley and Sons.
- Gan Z, Ellis PR, Schofield JD. (1995). Mini review: Gas cell stabilisation and gas retention in wheat bread dough. *J Cereal Sci* 21:215–30.
- Giannou V, Kessoglou V, Tzia C. (2003). Quality and safety characteristics of bread made from frozen dough. *Trends Food Sci Technol* 14:99– 108.
- Gibson GR & Roberfroid MB. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* 125: 1401–12.
- Gibson GR & Wang X (1994a). Enrichment of bifidobacteria from human gut contents by oligofructose using continuous culture. *FEMS Microbiology Letters* 118: 121–8.
- Gibson GR & Wang X (1994b). Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *Journal of Applied Bacteriology* 77: 412–20.
- Gibson GR, Beatty ER, Wang X et al. (1995). Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 108: 975–82
- Gibson, G. R., Beatty, E. R., Wang, X., and Cummings J. H. (1995). Selective stimulation of Bifidobacteria in the human colon by oligofructose and inulin, *Gastroenterology*, 108: 975–982
- Giczewska, A., & Borowska, J. (2003). Nutritional value of broad bean seeds. Part 1: Starch and fibre. *Food*, 47(2), 95–97.
- Glitsø, L.V. et al. (1999). Degradation of rye arabinoxylans in the large intestine of pigs, *J. Sci. Food Agric.*, 79, 961

- Glitsø, L.V., Jensen, B.B., and Knudsen, K.E.B.(2000). In vitro fermentation of rye carbohydrates including arabinoxylans of different structures, *J. Sci. Food Agric.*, 80, 1211
- Gobbetti M, Corsetti A, De Vincenzi S. (1995a). The sourdough microflora. Characterization of homofermentative lactic acid bacteria based on acidification kinetics and impedance tests. *Italian J Food Sci* 2:91–102.
- Gobbetti M, Corsetti A, De Vincenzi S. (1995b). The sourdough microflora. Characterization of heterofermentative lactic acid bacteria based on acidification kinetics and impedance tests. *Italian J Food Sci* 2:103–12.
- Gobbetti M, Corsetti A, Rossi J, La Rosa F, De Vincenzi S. (1994). Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of central Italy. *Italian J Food Sci* 1:85–94.
- Gobbetti M, De Angelis M, Arnault P, Tossut P, Corsetti A, Lavermicocca P. (1999). Added pentosans in breadmaking: fermentations of derived pentoses by sourdough lactic acid bacteria. *Food Microbiol* 16:409–18.
- Goni, I., Diaz-Rubio, M. E., Perez-Jimenez, J., & Saura-Calixto, F. (2009). Towards an updated methodology for measurement of dietary fiber, including associated polyphenols, in food and beverages. *Food Research International*, 42, 840–846.
- Goni, I., Garcia-Diz, E., Manas, E., & Saura-Calixto, F. (1996). Analysis of resistant starch: a method for foods and food products. *Food Chemistry*, 56, 445–449.
- Gordon, D. T., & Okuma, K. (2002). Determination of total dietary fiber in selected foods containing resistant maltodextrin by enzymatic-gravimetric method and liquid chromatography: Collaborative study. *Journal of AOAC International*, 85, 435–444.
- Grausgruber H, Sailer C, Ruckebauer P (2004) Khorasan wheat, Kamut and ‘Pharaonenkorn’: origin, characteristics and potential. *Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs* 55:75–80
- Grigelmo-Miguel, N., Carreras-Boladeras, E., & Martin-Belloso, O. (1999). Development of high-fruit-dietary-fibre muffins. *European Food Research and Technology*, 210, 123–128.
- Gruppen, H., Hamer, R.J. and Voragen, A.G.J. (1991). Barium hydroxide as a tool to extract pure arabinoxylans from water-insoluble cell wall material of wheat flour. *J. Cereal Sci.* 13, 275-290.
- Gudmudson, M., Eliasson, A.-C., Bengtsson, S., and Åman, P.(1991). The effects of watersoluble arabinoxylans on gelatinization and retrogradation of starch, *Starch/Starke*, 43, 5.
- Guillon, F., & Champ, M. (2000). Structural and physical properties of dietary fiber, and consequence of processing on human physiology. *Food Research International*, 33, 233–245.

- Gullo M, Romano AD, Pulvirenti A, Giudici P. (2003). *Candida humilis*- dominant species in sourdoughs for the production of durum wheat bran flour bread. *Intern J Food Microbiol* 80:55–9.
- H. Alexiou and A. Franck, (2008). Prebiotic inulin-type fructans:nutritional benefits beyond dietary fibre source. *BENEO-Orafti Nutrition Bulletin*, 33, 227–233
- Hallfrisch, J., Behall, K.M.(1997). Evaluation of foods and physiological responses to menus in which fat content was lowered by replacement with Oatrim. *Cereal Foods World* 43, 100–103.
- Hallfrisch, J., Schofield, D.J., Behall, K.M.(2003). Physiological responses of men and women to barley and oat extracts (NutrimX). II.Comparison of glucose and insulin responses. *Cereal Chemistry* 80, 80–83.
- Hammer K, Filatenko AA, Korzun V (2000) Microsatellite markers-a new tool for distinguishing diploid wheat species. *Genet Resour Crop Evol* 47:497–505
- Hammes WP, Ganzle MG. (1998). Sourdough breads and related products. In: Wood BJB, editor. *Microbiology of fermented foods*, Vol. 1. London: Blackie Academic and Professional. p 199–216.
- Hansen A, Lund B. (1987). Volatile compounds in rye sourdough. In: Martens M, Dalen GA, Russwurm H, editors. *Flavour science and technology*. New York: Wiley. p 43–49.
- Hansen A, Schieberle P. (2005). Generation of aroma compounds during sourdough fermentation: applied and fundamental aspects. *Trends Food Sci Technol* 16:85–94.
- Hansen HB, Andreassen MF, Nielsen MM, Larsen LM, Bach-Knudsen KE, Meyer AS, Christensen LP, Hansen A. (2002). Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. *Euro Food Res Technol* 214:33–42.
- Hansen, A., & Hansen, B. (1996). Flavour of sourdough wheat bread crumb. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, 202, 244–249.
- Hansen, A., Lund, B. & Lewis, M.J. (1989a). Flavour of sourdough rye bread crumb. *Lebensmittel Wissenschaft und Technologie* 22: 141-144.
- Hansen, H.B., Rasmussen, C.V., Knudsen, K.E.B., and Hansen, Å.(2003). Effects of genotype and harvest year on content and composition of dietary fibre in rye (*Secale cereale* L) grain, *J. Sci. Food Agric.*, 83, 76.
- Hans-Konrad Biesalski et Al.(2009). Bioactive compounds: Definition and assessment of activity. *Nutrition* 25, 1202–1205
- Haralampu, S. G. (2000). Resistant starch: A review of the physical properties and biological impact of RS3. *Carbohydrate Polymers*, 41, 285–292.

- Haralampu, S. G. (2000). Resistant starch: A review of the physical properties and biological impact of RS3. *Carbohydrate Polymers*, 41, 285–292.
- Härkönen, H., Pessa, E., Suortti, T. and Poutanen, K. (1997). Distribution and some properties of cell wall polysaccharides in rye milling fractions. *J. Cereal Sci.* 26, 95-104.
- Härkönen, H., Pessa, E., Suortti, T., and Poutanen, K.(1997). Distribution and some properties of cell wall polysaccharides in rye milling fractions, *J. Cereal Sci.*, 26, 95.
- Harmsen HJM, Raangs GC, Franks AH et al. (2002). The effect of the prebiotic inulin and the probiotic bifidobacterium longum on the fecal microflora of healthy volunteers measured by FISH and DGGE. *Microbial Ecology in Health and Disease* 14: 211–9.
- Harris, P.J., Chavan, R.R., and Ferguson, L.R.(2005). Production and characterization of two wheat-bran fractions: an aleurone-rich and a pericarp-rich fraction, *Mol. Nutr. Food Res.*, 49, 536
- Hashimoto, N., Ito, Y., Han, K. H., Shimada, K., Sekikawa, M., Topping, D. L., et al. (2006). Potato pulps lowered the serum cholesterol and triglyceride levels in rats. *Journal of Nutritional Science and Vitaminology*, 52, 445–450.
- Henry, R.J. (1985). A comparison of the non-starch carbohydrates in cereal grains. *J. Sci. Food Agric.* 36, 1243-1253.
- Henry, R.J.(1986). Genetic and environmental variation in the pentosan and -glucan contents in barley, and their relation to malting quality, *J. Cereal Sci.*, 4, 269.
- Henry, R.J.(1987). Pentosan and (1→3),(1→4)-β-glucan concentration in endosperm and wholegrain of wheat, barley, oats and rye, *J. Cereal Sci.*, 6, 253.
- Hernández, O., Emaldi, U., & Tovar, J. (2008). In vitro digestibility of edible films from various starch sources. *Carbohydrate Polymers*, 71, 648–655.
- Higgins, J. A. (2004). Resistant starch: Metabolic effects and potential health benefits. *Journal of the AOAC International*, 87(3), 761–768.
- Hilhorst, R. et al.(2002). Effects of xylanase and peroxidase on soluble and insoluble arabinoxylans in wheat bread dough, *J. Food Sci.*, 67, 497.
- Hizukuri S. (1986). Polymodal distribution of chain lengths of amylopectins and its significance. *Carbohydr Res* 147:342–7.
- Hoffman, W.F. and Gortner, R.A.(1927). The preparation and analysis of the various proteins of wheat flour with special reference to the globulin, albumin, and proteose fractions, *Cereal Chem.*, 4, 221.
- Hoffmann, R.A., Kamerling, J.P. and Vliegthart, J.F.G. (1992). Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.* 226, 303-311.

- Hofmann T, Schieberle P. (2000). Formation of aroma-active Strecker aldehydes by a direct oxidation of Amadori compounds. *J Agric Food Chem* 48:4301–5.
- Hopkins MJ, Cummings JH & Macfarlane GT (1998). Inter-species differences in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources. *Journal of Applied Microbiology* 85: 381–6.
- Hopkins, M.J. et al.(2003). Degradation of cross-linked and non-cross-linked arabinoxylans by the intestinal microbiota in children, *Appl. Environ. Microbiol.*, 69, 6354
- Hoseney RC, Wade P, Finley JW. (1988). Soft wheat products. In: Pomeranz Y, editor. *Wheat chemistry and technology*, Vol. 2. St. Paul, Minn.: American Association of Cereal Chemists. p 407–56.
- Howe, J. C., Rumpler, W. V., & Behall, K. M. (1996). Dietary starch composition and level of energy intake alter nutrient oxidation in ‘carbohydrate-sensitive’ men. *Journal of Nutrition*, 126, 2120–2129.
- Howlett, J.J.; Betteridge, V.A.; Champ, M.; Craig, S.A.S.; Meheust, A.; Jones, J.M.(2010). The definition of dietary fiber—Discussions at the Ninth Vahouny Fiber Symposium: Building scientific agreement. *Food Nutr. Res.* 54, doi:10.3402/fnr5405750 .
- Ishii, T. (1997). Structure and functions of feruloylated polysaccharides. *Plant Sci.* 127, 111-127.
- Izydorczyk, M.S. and Biliaderis, C.G.(1995). Cereal arabinoxylans: advances in structure and physicochemical properties, *Carbohydr. Polym.*, 28, 33.
- Izydorczyk, M.S. et al.(2005). The enrichment of Asian noodles with fiber-rich fractions derived from roller milling of hull-less barley, *J. Sci. Food Agric.*, 85, 2094.
- Izydorczyk, M.S., Macri, L.J., and MacGregor, A.W.(1998). Structure and physicochemical properties of barley non-starch polysaccharides. II. Alkali-extractable β -glucans and arabinoxylans, *Carbohydr. Polym.*, 35, 259.
- Jadhav, S.J., Lutz, S.E., Ghorpade, V.M., and Salunkhe, D.K.(1998). Barley: chemistry and value-added processing, *Crit. Rev. Food Sci.*, 38, 123.
- James M. Lattimer and Mark D. Haub.(2010). Effects of Dietary Fiber and Its Components on Metabolic Health. *Nutrients* 2, 1266-1289
- Jaskari, J. et al.(1998). Oat β -glucan and xylan hydrolysates as selective substrates for *Bifidobacterium* and *Lactobacillus* strains, *Appl. Microbiol. Biotechnol.*, 49, 175.
- Jeffries, T.W. (1990). Biodegradation of lignin-carbohydrate complexes. *Biodgradation*, 1, 163-176.
- Jenkins DJ, Wesson V, Wolever TM, et al. (1988). Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response. *BMJ* 297, 958–960 .

- Jenkins, A.L., Jenkins, D.J.A., Zdravkovic, U., Wursch, P., Vuksan, V. (2002). Depression of the glycaemic index by high levels of beta-glucan fibre in two functional foods tested in type 2 diabetes. *European Journal of Clinical Nutrition* 56, 622–628.
- Jenson I.(1998). Bread and baker's yeast. In: Wood BJB, editor. *Microbiology of fermented foods*. London: Blackie Academic and Professional. p 172–98.
- Jimenez-Escrig, A., & Sanchez-Muniz, F. J. (2000). Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research*, 20, 585–598.
- Jones JM (2008). Whole grains – issues and deliberations from the Whole Grain Task Force. *Cereal Foods World* 53,260–264 .
- Jones, J.(2000). Update on defining dietary fiber. *Cereal Foods World* 45, 219-220
- Kanauchi, M. and Bamforth, C.W.(2001). Release of β -glucan from cell walls of starchy endosperm of barley, *Cereal Chem.*, 78, 121.
- Katapodis, P. et al.(2003). Enzymatic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylans, *Eur. J. Nutr.*, 42, 55.
- Katrien, M.J. et al.(2000). Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria, *J. Agric. Food. Chem.*, 48, 1644.
- Kavita V, Varghese S, Chitra GR, Jamuna P. (1998). Effects of processing, storage time and temperature on the resistant starch of foods. *J Food Sci Technol* 35(4):299–304.
- Kavita V, Varghese S, Chitra GR, Jamuna P. (1998). Effects of processing, storage time and temperature on the resistant starch of foods. *J Food Sci Technol* 35(4):299–304.
- Keenan, M. J., Zhou, J., Mccutcheon, K. L., Raggio, A. M., Bateman, H. G., Todd, E., et al. (2006). Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity*, 14, 1523–1534.
- Kim, M. J., Choi, S. J., Shin, S. I., Sohn, M. R., Lee, C. J., Kim, Y., et al. (2008). Resistant glutarate starch from adlay: Preparation and properties. *Carbohydrate Polymers*, 74, 787–796.
- Kleessen B, Hartmann L & Blaut M (2001). Oligofructose and longchain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora. *British Journal of Nutrition* 86: 291–300.
- Kleessen, B., Sykura, B., Zunft, H. J., and Blaut, M.(1997). Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons, *Am. J. Clin. Nutr.*, 65: 1397– 1402.
- Klopfenstein, C.F.(1988). The role of cereal beta-glucans in nutrition and health, *Cereal Foods*

- World, 33, 865.
- Knuckles, B.E., Hudson, C.A., Chiu, M.M.(1997b). Effect of beta glucan barley fractions in high fibre bread and pasta. *Cereal Foods World* 42, 94–99.
- Knuckles, B.E., Hudson, C.A., Chiu, M.M., and Sayre, R.N.(1997). Effect of β -glucan barley fractions in high-fiber bread and pasta, *Cereal Foods World*, 42, 94.
- Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, et al. (2002).A high-resolution recombination map of the human genome. *Nat Genet*;6:10.
- Kruse, H. P., Kleessen, B., and Blaut, M. (1999). Effects of inulin on faecal bifidobacteria in human subjects. *Br. J. Nutr.* 82:375-382.
- Kulp K, Lorenz K. (2003). *Handbook of dough fermentations*. New York: Marcel Dekker Inc. p 1–21.
- Kuniak, L., & Marchessault, R. H. (1972). Study of the crosslinking reaction between epichlohydrin and starch. *Die Starke*, 4, 110–116.
- Labat, E., Rouau, X., and Morel, M.-H.(2002). Effect of flour water-extractable pentosans on molecular associations in gluten during mixing, *Lebensm. Wiss. Technol.*, 35, 185.
- Lang R & Jebb SA (2003). Who consumes whole grains, and how much? *Proc Nutr Soc* 62, 123 –127
- Langlands SJ, Hopkins MJ, Coleman N et al. (2004). Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. *Gut* 53: 1610–6.
- Larrauri, J. A. (1999). New approaches in the preparation of high dietary fiber powders from fruit by-products. *Trends in Food Science and Technology*, 10, 3–8.
- Laurikainen, T., Harkonen, H., Autio, K., & Poutanen, K. (1998). Effects of enzymes in fiber-enriched baking. *Journal of the Science of Food and Agriculture*, 76, 239–249.
- Lazaridou, A. and Biliaderis, C.G.(2004). Cryogelation of cereal β -glucans: structure and molecular size effects, *Food Hydrocolloids*, 18, 933.
- Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2007). Cereal b-glucans: Structures, physical properties, and physiological functions. In C. G. Biliaderis & M. S. Izydorczyk (Eds.), *Functional food carbohydrates* (pp. 1–72). Boca Raton: CRC Press, Taylor & Francis Group.
- Lazaridou, A., Biliaderis, C.G., Micha-Screttas, M., and Steele, B.R.(2004). A comparative study on structure-function relations of mixed linkage (1→3),(1→4) linear β -D-glucans, *Food Hydrocolloids*, 18, 837.
- Lee, C.J., Horsley, R.D., Manthey, F.A., and Schwarz, P.B.(1997). Comparisons of β -glucan content of barley and oat, *Cereal Chem.*, 74, 571.
- Lee, S., Prosky, L., & DeVries, J. (1992). Determination of total, soluble, and insoluble dietary fiber

- in foods: Enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. *Journal of the Association of Official Analytical Chemists*, 75, 395–416.
- Lempereur, I., Rouau, X., and Abecassis, J.(1997) Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (*Triticum durum* L.) grain and its milling fractions, *J. Cereal Sci.*, 25, 103.
- Leu, R. K., Hu, Y., & Young, G. P. (2002). Effects of resistant starch and nonstarch polysaccharides on colonic luminal environment and genotoxin-induced apoptosis in the rat. *Carcinogenesis*, 23(5), 713–719.
- Lewis HB (1912) The value of inulin as a foodstuff. *Journal of the American Medical Association* 58: 1176–7.
- Lewis S, Burmeister S & Brazier J .(2005). Effect of the prebiotic oligofructose on relapse of *Clostridium difficile*-associated diarrhea: a randomized, controlled study. *Clinical Gastroenterology and Hepatology* 3: 442–8.
- Liljeberg H, Akerberg A, Bjorck I. (1996). Resistant starch formation in bread as influenced by choice of ingredients or baking conditions. *Food Chem* 56(4):389–94.
- Lindsay JO, Whelan K, Stagg AJ et al. (2006). Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease. *Gut* 55: 348–55.
- Liu, R., & Xu, G. (2008). Effects of resistant starch on colonic preneoplastic aberrant crypt foci in rats. *Food and Chemical Toxicology*, 46, 2672–2679.
- Lopez, H.W. et al.(1999). Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats, *J. Nutr. Biochem.*, 10, 500
- Lu, Z.X. et al.(2000). Arabinoxylan fiber, a by-product of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects, *Am. J. Clin. Nutr.*, 71, 1123.
- Lund B, Hansen A, Lewis MJ. (1987). The influence of yeast and sourdough fermentation on volatile compounds in rye bread crumb. In: Munck L, editor. *Cereal science and technology*. Copenhagen: proceedings from 23. Nordic Cereal Congress, The Danish Cereal Society. p 185–95.
- Lunn, J., & Buttriss, J. L. (2007). Carbohydrates and dietary fibre. *Nutrition Bulletin*, 32, 21–64
- Lunn, J., & Buttriss, J. L. (2007). Carbohydrates and dietary fibre. *Nutrition Bulletin*, 32, 21–64.
- MacGregor, A.W. and Fincher, G.B.(1993). Carbohydrates of the barley grain, in *Barley: Chemistry and Technology*, MacGregor, A.W. and Bhatta, R.S., Eds., American Association of Cereal Chemists, St. Paul, MN, p. 73 .
- Manas, E., Bravo & Saura-Calixto, F. (1994). Source of error in dietary fiber analysis. *Food*

- Chemistry, 50, 331–342.
- Marconi, E., Graziano, M., and Cubadda, R.(2000). Composition and utilization of barley pearling by-products for making functional pastas rich in dietary fiber and β -glucans, *Cereal Chem.*, 77, 133.
- Marconi, E., Graziano, M., and Cubadda, R.(2000). Composition and utilization of barley pearling by-products for making functional pastas rich in dietary fiber and β -glucans, *Cereal Chem.*, 77, 133.
- Mares, D.J. and Stone, B.A.(1973). Studies on wheat endosperm. I. Chemical composition and ultrastructure of the cell walls, *Austr. J. Biol. Sci.*, 26, 793.
- Markwalder, H.U. and Neukom, H. (1976). Diferulic acid as possible crosslink in hemicelluloses from wheat germ. *Phytochemistry*, 15, 836-837.
- Matz SA. (1996). Chemistry and technology of cereals as food and feed. 2nd ed. New York: Van Nostrand Reinhold.
- McCleary, B. V., & Monaghan, D. A. (2002). Measurement of resistant starch. *Journal of AOAC International*, 85, 665–675
- McCleary, B. V., Jonathan, W., De Vries, J. W., Rader, J. I., Cohen, G., Prosky, L., et al. (2010). Determination of total dietary fiber (CODEX definition) by enzymaticgravimetric method and liquid chromatography: Collaborative study. *Journal of AOAC International*, 93, 221–233
- McCleary, B.V.(1986). Enzymatic modification of plant polysaccharides, *Int. J. Biol. Macromol.*, 8, 349
- McNeil, M., Alberheim, P. Taiz, L. and Jones, R.L. (1975). Structure of plant cell walls. VII. Barley aleurone cells. *Plant Physiol.* 55, 64-68.
- Menne E, Guggenbuhl N & Roberfroid M (2000). Fn-type chicory inulin hydrolysate has a prebiotic effect in humans. *Journal of Nutrition* 130: 1197–9.
- Michniewicz, J., Biliaderis, C.G., and Bushuk, W.(1991). Effect of added pentosans on some physical and technological characteristics of dough and gluten, *Cereal Chem.*, 68, 252
- Mikulíková, D., Masár, S., & Kraic, J. (2008). Biodiversity of legume health-promoting starch. *Starch*, 60, 426–432.
- Mikušová, L., Šturdík, E., Mošovská, S., Brindzová, L., & Mikulajová, A. (2009). Development of new bakery products with high dietary fibre content and antioxidant activity for obesity prevention. In *Proceedings of 4th international dietary fibre conference* (p. 185). Vienna, Austria: International association for cereal science and technology (ICC).
- Miller, S.S. and Fulcher, R.G.(1994). Distribution of (1→3),(1→4)- β -D-glucan in kernels of oats and

- barley using microspectrofluorometry, *Cereal Chem.*, 71, 64.
- Miller, S.S., Wood, P.J., Pietrzak, L.N., and Fulcher, R.G.(1993). Mixed linkage beta glucans, protein content, and kernel weight in *Avena* species, *Cereal Chem.*, 70, 231.
- Mohamed Elleuch, Dorothea Bedigian, Olivier Roiseux, Souhail Besbes, Christophe Blecker, Hamadi Attia.(2011).Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry* 124, 411–421
- Morita H, Taguchi J, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, et al.(1997). Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease. *Circulation*; 95:2032–6.
- Moure, A., Gullon, P., Dominguez, H. and Parajo, J.C. (2006). Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. *Process Biochem.* 41, 1913-1923.
- Muir JG, O’Dea K. (1992). Measurement of resistant starch. Factors affecting the amount of starch escaping digestion in vitro. *Am J Clin Nutr* 56:123–7.
- National Council on Nutrition and Physical Activity and the Institute for Nutrition Research (1998). Development of the Norwegian Diet. Oslo: University of Oslo .
- Newman, R.K. and Newman, C.W.(1991) Barley as a food grain, *Cereal Foods World*, 36, 800,.
- Newman, R.K. et al.(1992). Comparison of the cholesterol-lowering properties of whole barley, oat bran, and wheat red dog in chicks and rats, *Cereal Chem.*, 69, 240.
- Newman, R.K., Ore, K.C., Abbott, J., and Newman, C.W.(1998). Fiber enrichment of baked products with a barley milling fraction, *Cereal Foods World*, 43, 23.
- Nilsson AC, Ostman EM, Holst JJ, et al. (2008). Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast . *J Nutr* 138, 732–739.
- Normand, F. L., Ory, R. L., & Mod, R. R. (1987). Binding of bile acids and trace minerals by soluble hemicelluloses of rice. *Food Technology*, 41, 86–99.
- Nugent, A. P. (2005). Health properties of resistant starch. British Nutrition Foundation, *Nutrition Bulletin*, 30, 27–54.
- Ohkuma, K., Matsuda, I., Katta, Y., & Tsuji, K. (2000). New method for determining total dietary fiber by liquid chromatography. *Journal of AOAC International*, 83, 1013–1019.
- Olson, A., Gray, G. M., & Chiu, M. C. (1987). Chemistry and analysis of soluble dietary fibre. *Food Technology*, 41, 71–80.

- Ottogalli G, Galli A, Foschino R. (1996). Italian bakery products obtained with sourdough: characterization of the typical microflora. *Adv Food Sci* 18:131–44.
- Parchure AA, Kulkarni PR. (1997). Effect of food processing treatments on generation of resistant starch. *Intl. J Food Sci Nutr* 48:257–60.
- Parkkonen, T., Harkonen, H., and Autio, K.(1994). Effect of baking on the microstructure of rye cell walls and protein, *Cereal Chem.*, 71, 58.
- Pedersen J. (1994). Insects: identification, damage and detection. In: Sauer DB, editor. *Storage of Cereal Grains*. St. Paul, Minn.: American Association Cereal Chemists. p 435–89.
- Pérez-Jiménez J, Arranz S, Saura-Calixto F. (2009). Proanthocyanidin content in foods is largely underestimated in the literature data. An approach to quantification of the missing proanthochyanidins. *Food Res Internat*, 42; 1381-1388
- Pitkänen, L., Virkki, L., Tenkanen, M. and Tuomainen, P. (2009). Comprehensive multidetector HPSEC study on solution properties of cereal arabinoxylans in aqueous and DMSO solutions. *Biomacromolecules* 10, 1962-1969.
- Posner ES.(2000). Wheat. In: Kulp K, Ponte JG, editors. *Handbook of cereal science and technology*. New York: Marcel Dekker. p 130.
- Prattala R, Helasoja V & Mykkanen H (2007). The consumption of rye bread and white bread as dimensions of health lifestyles in Finland. *Public Health Nutr* 4, 813–819 .
- Prosky, L., Asp, G. N., Scheweizer, T. F., de Vries, J. W., & Furda, I. (1992). Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. *Journal of the Association of Analytical Chemistry International*, 75, 360–367.
- Prosky, L., Asp, N.-G., Scheweizer, T. F., DeVries, J. W., & Furda, I. (1988). Determination of insoluble and soluble, and total dietary fibre in foods and food products: Interlaboratory study. *Journal of the Association of Official Analytical Chemists*, 71, 1017–1023.
- Quemener, B., Thibault, J. F., & Coussement, P. (1994). Determination of inulin and oligofructose in food products and integration in the AOAC method for the measurement of total dietary fibre. *Lebensmittel-Wissenschaft und Technologie*, 27, 125–132.
- Quinn RM .(1999). Kamut ® : ancient grain, new cereal Perspectives on New Crops and New Uses 182 – 183 Janick J Alexandria, VA ASHS Press
- R. Mujoo and P.K.W. NG.(2003). Physicochemical Properties of Bread Baked from Flour Blended with Immature Wheat Meal Rich in Fructooligosaccharides. *Journal of Food Science*:68, 8.
- Rabe E, Sievert D. (1992). Effects of baking, pasta production and extrusion cooking on formation of resistant starch. *Eur J Clin Nutr* 46(Suppl):S105–6.

- Raben, A., Andersen, K., & Karberg, M. A. (1997). Acetylation of or B-cyclodesetrin addition to potato starch: Beneficial effect on glucose metabolism and appetite sensations. *American Journal of Clinical Nutrition*, 66, 304–314.
- Raben, A., Tagliabue, A., Christensen, N. J., Madsen, J., Holst, J. J., & Astrup, A. (1994). Resistant starch: The effect on postprandial glycemia, hormonal response and satiety. *American Journal of Clinical Nutrition*, 60, 544–551.
- Ranganathan, S., Champ, M., Pechard, C., Blanchard, P., Nguyen, M., Colonna, P., et al. (1994). Comparative study of the acute effects of resistant starch and dietary fibres on metabolic indexes in men. *American Journal of Clinical Nutrition*, 59, 879–883.
- Rao VA (2001). The prebiotic properties of oligofructose at low intake levels. *Nutrition Research* 21: 843–8.
- Ratnayake, W. S., & Jackson, D. S. (2008). Thermal behavior of resistant starches RS 2, RS 3, and RS 4. *Journal of Food Science*, 73(5), 356–366.
- Ratnayake, W. S., & Jackson, D. S. (2008). Thermal behavior of resistant starches RS 2, RS 3, and RS 4. *Journal of Food Science*, 73(5), 356–366.
- Reader, D., Johnson, M. L., Hollander, P., & Franz, M. (1997). Response of resistant starch in a food bar vs. two commercially available bars in persons with type II diabetes mellitus. *Diabetes*, 46(1), 254.
- Rehman S, Paterson A, Piggott JR. (2006). Flavour in sourdough breads: a review. *Trends Food Sci Technol* 17:557–66.
- Rehman, Z.-U., Islam, M., & Shah, W. H. (2003). Effect of microwave and conventional cooking on insoluble dietary fibre components of vegetables. *Food Chemistry*, 80(2), 237–240.
- Ring SG, Gee JM, Whittam M, Orford, P, Johnson I. (1988). Resistant Starch. Its chemical form in foodstuffs and effect on digestibility in vitro. *Food Chem* 28:97–109.
- Roben, A., Andersen, K., & Karberg, M. A. (1997). Acetylation of or Bcyclodesetrin addition to potato starch: Beneficial effect on glucose metabolism and appetite sensations. *American Journal of Clinical Nutrition*, 66, 304–314.
- Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, Wolvers D, Watzl B, Szajewska H, Stahl B, et al. (2010). Prebiotic effects: Metabolic and health benefits. *Br J Nutr* 104(Suppl 2):S1–S63.
- Roberfroid MB (2005). Introducing inulin-type fructans. *British Journal of Nutrition* 93 (Suppl. 1): S13–25.
- Roberfroid MB, Cumps J & Devogelaer JP .(2002). Dietary chicory inulin increases whole-body

- bone mineral density in growing male rats. *Journal of Nutrition* 132: 3599–602.
- Roberfroid MB, Gibson GR & Delzenne N (1993). The biochemistry of oligofructose, a nondigestible fiber: an approach to calculate its caloric value. *Nutrition Reviews* 51: 137–46.
- Roberfroid MB, Van Loo JAE & Gibson GR (1998). The bifidogenic nature of chicory inulin and its hydrolysis products. *Journal of Nutrition* 128: 11–9.
- Roberfroid, M. B. (2000). Prebiotics and probiotics: Are they functional foods? *American Journal of clinical Nutrition*, 71(6), 1682–1687.
- Rochfort, S., & Panozzo, J. (2007). Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry*, 55, 7981–7994.
- Rodríguez, S. L., Islas, J. J., Agama, E., Tovar, J., & Bello, L. A. (2008). Characterization of a fibre-rich powder prepared by liquefaction of unripe banana flour. *Food Chemistry*, 107, 1515–1521.
- Rodriguez-Quijano M, Lucas R, Ruiz M, Giraldo P, Espi' A, Carrillo JM (2010) Allelic variation and geographical patterns of prolamins in the USDA-ARS Khorasan wheat collection. *Crop Sci* 50:2383–2391 germplasm
- Roehrig, K. L. (1988). The physiological effects of dietary fiber. *Food Hydrocolloids*, 2, 1–18.
- Rondini, L. et al. (2004). Bound ferulic acid from bran is more available than the free compound in rat, *J. Agric. Food Chem.*, 52, 4338.
- Rossi J. (1996). The yeasts in sourdough. *Adv Food Sci* 18:201–11.
- Rothe M, Ruttloff H. (1983). Aroma retention in modern bread production. *Nahrung* 27:505–12.
- Saastamoinen, M., Plaami, S., and Kumpulainen, J. (1989). Pentosan and beta-glucan content of Finnish winter rye varieties as compared with rye grain from several countries, *J. Cereal Sci.*, 10, 199.
- Saastamoinen, M., Plaami, S., and Kumpulainen, J. (1992). Genetic and environmental variation in β -glucan content of oats cultivated or tested in Finland, *J. Cereal Sci.*, 16, 279.
- Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch – A review. *Comprehensive Reviews in Food Science and Food Safety*, 5, 1–17.
- Salmenkallio-Marttila M, Katina K, Autio K. (2001). Effect of bran fermentation on quality and microstructure of high-fibre wheat bread. *Cereal Chem* 78:429–35.
- Salminen SJ, Gueimonde M & Isoauri E (2005). Probiotics that modify disease risk. *Journal of Nutrition* 135: 1294–8.
- Sangnark, A., & Noomhorm, A. (2004). Chemical, physical and baking properties of dietary fiber prepared from rice straw. *Food Research International*, 37, 66–74.
- Sanz, T., Salvador, A., & Fiszman, S. M. (2008a). Resistant starch (RS) in battered fried products:

- Functionality and high-fibre benefit. *Food Hydrocolloids*, 22, 543–549.
- Sanz, T., Salvador, A., & Fiszman, S. M. (2008b). Evaluation of four types of resistant starch in muffin baking performance and relationship with batter rheology. *European Food Research & Technology*, 227, 813–819.
- Sanz, T., Salvador, A., Baixauli, R., & Fiszman, S. M. (2009). Evaluation of four types of resistant starch in muffins. II. Effects in texture, colour and consumer response. *European Food Research & Technology*, 229(2), 197–204.
- Schaller, D. A. (1976). Analysis of dietary fiber. *Food Production and Development*, 11, 70–72.
- Schieberle, P. 1996. Intense aroma compounds – useful tools to monitor the influence of processing and storage on bread aroma. *Advances in Food Science (CMTL)* 18: 237-244.
- Schneeman, B. O. (1987). Soluble and insoluble fibre-different physiological responses. *Food Technology*, 41, 81–82.
- Schnyder H, Ehses U, Bestajovsky J, Mehrhoff R, Kuhbauch W. (1988). Fructan in wheat kernels during growth and compartmentation in the endosperm and pericarp. *Plant Physiol* 132:333–8.
- Scholtens PAM, Alles MS, Willemsen LEM et al. (2006). Dietary fructo-oligosaccharides in healthy adults do not negatively affect faecal cytotoxicity: a randomised, double-blind, placebocontrolled crossover trial. *British Journal of Nutrition* 95: 1143–9.
- Scholz-Ahrens K, Açı Y & Schrezenmeir J. (2002). Effect of oligofructose or dietary calcium on repeated calcium and phosphorus balances, bone mineralization and trabecular structure in ovariectomized rats. *British Journal of Nutrition* 88: 365–77.
- Scholz-Ahrens, E., Ade, P., Marten, B., Weber, P., Timm, W., Yahya, A., et al. (2007). Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *Journal of Nutrition*, 137, 838–846.
- Schooneveld-Bergmans, M.E.F., van Dijk, Y.M., Beldman, G., and Voragen, A.G.J. (1999). Physicochemical characteristics of wheat bran glucuronoarabinoxylans, *J. Cereal Sci.*, 29, 49.
- Schulz A. (1972). Der Einfluss organischer Säuren auf die Vergärung verschiedener Z. Getr Mehl Brot 26:129–33.
- Schweizer, TF, Anderson H, Lankilde AM, Reimann S, Torsdottir I. (1990). Nutrients excreted in ileostomy effluents after consumption of mixed diets with beans or potatoes II. Starch, dietary fiber and sugars. *Eur J Clin Nutr* 44:567–75.
- Schweizer, T. F., Andersson, H., Langkilde, A. M., Reimann, S., and Torsdottir, I., Nutrients excreted in ileostomy effluents after consumption of mixed diets with beans or potatoes. II. Starch, dietary fibre and sugars, *Eur. J. Clin. Nutr.*, 1990; 44: 567–575.

- Seibel W. (1983). Anrechnung von Brot und Backverhalten. *Getreide Mehl und Brot* 12:377–79.
- Sharma, A., Yadav, B. S., & Ritika (2008). Resistant starch: Physiological roles and food applications. *Food Reviews International*, 24, 193–234.
- Sharma, A., Yadav, B. S., & Ritika (2008). Resistant starch: Physiological roles and food applications. *Food Reviews International*, 24, 193–234.
- Shimada, M., Mochizuki, K., & Goda, T. (2008). Dietary resistant starch reduces levels of glucose-dependent insulinotropic polypeptide mRNA along the jejunum–ileum in both normal and type 2 diabetics rats. *Bioscience, Biotechnology and Biochemistry*, 72(8), 2206–2209.
- Siljestrom M, Asp NG. (1985). Resistant starch formation during baking. Effect of baking time and temperature and variation in the recipe. *Z Lebensm Unters Forsch* 4:1–18.
- Siljestrom M, Bjorck I. (1990). Digestible and undigestible carbohydrates in autoclaved legumes, potatoes and corn. *Food Chem* 38:145–52.
- Siljestrom M, Eliasson AC, Asp NG. (1989). Characterisation of resistant starch from autoclaved wheat starch. *Starch/Starke* 41:147–51.
- Simonato B, Pasini G, Giannattasio M, Curioni A (2002) Allergenic potential of Kamut wheat. *Allergy* 57:653–654
- Sjöström, E. (1981). *Wood chemistry. Fundamentals and applications*. Academic Press, Inc., New York,
- Slavin, J. (2004). Whole grains and human health. *Nutrition Research Reviews*, 17, 99–110.
- Slavin, J.L.; Savarino, V.; Parades-Diaz, A.; Fotopoulos, G.(2009). A review of the role of soluble fiber in health with specific reference to wheat dextrin. *J. Int. Med. Res.* 37, 1–17 .
- Southgate, D. A. T., Hudson, G. J., & Englyst, H. (1978). The analysis of dietary fibre – the choices for the analyst. *Journal of the Science of Food and Agriculture*, 29, 979–989.
- Spicher G, Schroder R. (1978). Die Mikroflora des Sauerteiges. IV. Mitteilung: untersuchungen u“ber die Art der in “Reinzuchtsauern” anzutreffenden sta“bchenfo“rmigen Milchsau“urebakterien (Genus *Lactobacillus* Beijerinck). *Z Lebensm Unters Forsch* 167:342–54.
- Spicher G, Stephan H. (1999). *Handbuch sauerteig, biologie, biochemie, technologie*. 5th ed. Hamburg: Behr’s Verlag.
- Spicher G. (1983). Baked goods. In: Rehm JH, Reed G, editors.
- Srivastava AK, Sudha ML, Baskaran V, et al. (2007) . Studies on heat stabilized wheat germ and its influence on rheological characteristics of dough. *Eur Food Res Technol* 224, 365 –372.
- Stallknecht GF Gilbertson KM Ranney JE .(1996). Alternative wheat cereals as food grains: Einkorn, Emmer, Spelt, Kamut, and Triticale *Progress in New Crops* 156 – 170 Janick J Alexandria, VA

- Sternemalm, E., Höije, A. and Gatenholm, P. (2008). Effect of arabinose substitution on the material properties of arabinoxylan films. *Carbohydr. Res.* 343, 753-757.
- Stojceska, V., Ainsworth, P., Plunkett, A., & Ibanoglu, S. (2010). The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. *Food Chemistry*, 121, 156–164.
- Storsley, J.M. et al.(2003). Structure and physicochemical properties of β -glucans and arabinoxylans isolated from hull-less barley, *Food Hydrocolloids*, 17, 831.
- Succi M, Reale A, Andrighetto C, Lombardi A, Sorrentino E, Coppola R. (2003). Presence of yeasts in Southern Italian sourdoughs from *Triticum aestivum* flour. *FEMS Microbiol Lett* 225:143–8.
- Symons, L.J., Brennan, C.S., (2004b). The physicochemical and nutritional evaluation of wheat breads supplemented with (1/3) (1/4)- b-D-glucan rich fractions from barley. *Journal of Food Science* 69, 463–467.
- Tagliabue, A., Raben, A., Heijnen, M. L., Deurenberg, P., Pasquali, E., & Astrup, A. (1995). The effect of raw potato starch on energy expenditure and substrate oxidation. *American Journal of Clinical Nutrition*, 61, 1070–1075.
- Taper HS & Roberfroid MB (1999). Influence of inulin and oligofructose on breast cancer and tumour growth. *J Nutr* 129, 1488S–1491S.
- Taper HS & Roberfroid MB (2000). Inhibitory effect of dietary inulin or oligofructose on the development of cancer metastases. *Anticancer Res* 20, 4291–4294.
- Taper HS & Roberfroid MB (2002a). Non-toxic potentiation of cancer radiotherapy by dietary oligofructose and inulin. *Anticancer Res* 22, 3319–3324.
- Taper HS & Roberfroid MB (2002b). Inulin/oligofructose, and anticancer therapy. *Br J Nutr* 87, Suppl. 2, S283–S286.
- Taper HS, Delzenne NM & Roberfroid MB (1997). Growth inhibition of transplantable mouse tumors by non-digestible carbohydrates. *Int J Cancer* 71, 1109–1112.
- Taper HS, Lemort C & Roberfroid MB (1998). Inhibition effect of dietary inulin and oligofructose on the growth of transplantable mouse tumor. *Anticancer Res* 18, 4123–4126.
- Tapsell, L. C. (2004). Diet and metabolic syndrome: Where does resistant starch fit in? *Journal of the Association of Analytical Chemists International*, 87(3), 756–760.
- Tharanathan, R. N. (2002). Food-derived carbohydrates: Structural complexity and functional diversity. *Critical Reviews in Biotechnology*, 22(1), 65–84.
- Tharanathan, R. N., & Mahadevamma, S. (2003). Grain legumes: A boon to human nutrition. *Trends*

- in Food Science & Technology, 14, 507–518.
- Theander, O., Aman, P., Westerlund, E., Andersson, R., & Pettersson, D. (1995). Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the Uppsala method): Collaborative study. *Journal of AOAC International*, 78, 1030–1044.
- Thiele C, Ganzle MG, Vogel RF. (2002). Contribution of sourdough lactobacilli, yeast, and cereal enzymes to the generation of amino acids in dough relevant for bread flavor. *Cereal Chem* 79:45–51.
- Thompson DB.(2000). On the non-random nature of amylopectin branching. *Carbohydr Polym* 43:223–39.
- Topping D (2007). Cereal complex carbohydrates and their contribution to human health. *J Cereal Sci* 46, 220–229
- Topping, D. L., Anthony, M. F., & Bird, F. (2003). Resistant starch as prebiotic and synbiotic: State of the art. *Proceedings of the Nutrition Society*, 62, 171–176.
- Tosh, S.M., Wood, P.J., Wang, Q., and Weisz, J.(2004). Structural characteristics and rheological properties of partially hydrolyzed oat β -glucan: the effects of molecular weight and hydrolysis method, *Carbohydr. Polym.*, 55, 425
- Tovar J, Melito C. (1996). Steam-cooking and dry heating produce resistant starch in legumes. *J Agric Food Chem* 44(9):2642–5.
- Truswell AS (2002). Cereal grains and coronary heart disease. *Eur J Clin Nutr* 56, 1–14 .
- Truwel, A. S. (1992). Glycemic index of foods. *European Journal of Clinical Nutrition*, 46(2), 91–101.
- Tuohy KM, Finlay RK, Wynne AG et al. (2001). A human volunteer study on the prebiotic effects of HP-inulin – faecal bacteria enumerated using fluorescent in situ hybridisation (FISH). *Anaerobe* 7: 113–8.
- Vaikousi, H. and Biliaderis, C.G.(2005). Processing and formulation effects on rheological behavior of barley β -glucan aqueous dispersions, *Food Chem.*, 91, 505.
- Vaikousi, H., Biliaderis, C.G., and Izydorczyk, M.S.(2004). Solution flow behavior and gelling properties of water-soluble (1→3),(1→4) β -D -glucans varying in molecular size, *J. Cereal Sci.*, 39, 119
- Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H. & Smits, G. (1995) On the presence of inulin and oligofructose as natural ingredients in the western diet. *Crit. Rev. Food Sci. Nutr.* 35, 525–552
- Van Soest, P. J. (1963). Use of detergents in the analysis of fibrous feeds. II. Determination of plant

- cell-wall constituents. *Journal of the Association of Official Analytical Chemistry*, 48, 829–835.
- Van Soest, P. J., & Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Official Analytical Chemistry*, 50, 50–55.
- Varum, K.M. and Smidsrod, O.(1988)- Partial chemical and physical characterization of (1→3),(1→4) linear β -D-glucans from oat (*Avena sativa* L.) aleurone, *Carbohydr. Polym.*, 9, 103.
- Vasanthan, T., Gaosong, J., Yeung, J., & Jihong, L. (2002). Dietary fiber profile of barley as affected by extrusion cooking. *Food Chemistry*, 77, 35–40.
- Vavilov NI .(1951). The origin, variation, imunity and breeding of cultivated plants. *Chronica Botanica* 13. *Chronica Botanica Co.*, Waltham
- Vinkx, C.J.A. and Delcour, J.A.(1996). Rye (*Secale cereale* L.) arabinoxylans: a critical review, *J. Cereal Sci.*, 24, 1.
- Visser RGF, Suurs LCJM, Bruinenberg PM, Bleeker I, Jacobsen E. (1997). Comparison between amylose-free and amylose containing potato starches. *Starch/Stärke* 49:438–43.
- Vogel RF, Ehrmann MA, Ganzle MG. (2002). Development and potential of starter lactobacilli resulting from exploration of the sourdough ecosystem. *Antonie van Leeuwenhoek* 81:631–38.
- Wang X & Gibson GR (1993). Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Bacteriology* 75: 373–80.
- Wang, M. et al.(2003). Effect of water unextractable solids on gluten formation and properties: mechanistic considerations, *J. Cereal Sci.*, 37, 55.
- Wang, M., Hamer, R.J., Vliet von, T., and Oudgenoeng, G.(2002). Interaction of water extractable pentosans with gluten protein: effect of dough properties and gluten quality, *J. Cereal Sci.*, 36, 25.
- Wang, M., Vliet von, T., and Hamer, R.J.(2004). How gluten properties are affected by pentosans, *J. Cereal Sci.*, 39, 395.
- Warrand, J. et al.(2005). Flax (*Linum usitatissimum*) seed cake: a potential source of high molecular weight arabinoxylans? *J. Agric. Food Chem.*, 53, 1449.
- Webster, F.H.(1986). Oat utilization: past, present, and future, in *Oats: Chemistry and Technology*, Webster, F.H., Ed., American Association of Cereal Chemists, St. Paul, MN, p. 426.
- Weightman, R. M., Renard, C. M. G. C., Gallant, D. J., & Thibault, J.-F. (1995). Structure and properties of the polysaccharides from pea hulls II. Modification of the composition and physico-chemical properties of pea hulls by chemical extraction of the constituent

- polysaccharides. *Carbohydrate Polymers*, 26,121–128.
- Welsh S, Shaw A & Davis C (1994). Achieving dietary recommendations – whole-grain foods in the food guide pyramid. *Crit Rev Food Sci Nutr* 34, 441–451 .
- Wepner, B., Berghofer, E., Miesenberger, E., & Tiefenbacher, K. (1999). Citrate starch: Application as resistant starch in different food systems. *Starch*, 51(10),354–361.
- Westerlund E, Theander O, Andersson R, Aman P. (1989). Effects of baking on polysaccharides in white bread fractions. *J Cereal Sci* 10(2):149–56.
- Wood, P.J. et al.(1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load, *Br. J. Nutr.*, 72, 731.
- Wood, P.J. et al.(2003). Structure of (1→3)(1→4)- β -D-glucan in waxy and nonwaxy barley, *Cereal Chem.* 80, 329
- Wood, P.J.(1986). Oat β -glucan: structure, location, and properties, in *Oats: Chemistry and Technology*, Webster, F.H., Ed., American Association of Cereal Chemists, St. Paul, MN, p. 121.
- Wood, P.J.(1993). Physicochemical characteristics and physiological properties of oat (1→3),(1→4)- β -D-glucan, in *Oat Bran*, Wood, P.J., Ed., American Association of Cereal Chemists, St. Paul, MN, p. 83 .
- Wood, P.J., Siddiqui, I.R., and Paton, D.(1978). Extraction of high viscosity gums from oats, *Cereal Chem.*, 55, 1038
- Wood, P.J., Weisz, J., and Blackwell, B.A.(1991). Molecular characterization of cereal β - D-glucans. Structural analysis of oat β -D-glucan and rapid structural evaluation of β -D-glucans from different sources by high-performance liquid chromatography of oligosaccharides released by lichenase, *Cereal Chem.*, 68, 31.
- Woodward, J.R., Fincher, G.B., and Stone, B.A. (1983).Water-soluble (1→3),(1→4) linear β -D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure, *Carbohydr. Polym.*, 3, 207.
- Woodward, J.R., Phillips, D.R., and Fincher, G.B.(1988) Water-soluble (1→3),(1→4) linear β -D-glucans from barley (*Hordeum vulgare*) endosperm. IV. Comparison of 40oC and 65oC soluble fractions, *Carbohydr. Polym.*, 8, 85
- Wu HC, Sarko A. (1978). The double helical molecular structure of crystalline A-amylose. *Carbohydr Res* 61:7.
- Wu, Y.V., Stringfellow, A.C., and Inglett, G.E.(1994). Protein- and β -glucan enriched fractions from high-protein, high- β -glucan barleys by sieving and air classification, *Cereal Chem*, 71, 220.

- Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnland, J.R., Schneeman, B.O.(1997). Effect of barleyb-glucan in durum wheat pasta on human glycaemic response. *Cereal Chemistry* 74, 293–296.
- Zelevnak, K. and Hoseney, R.C. (1986).The role of water in the retrogradation of wheat starch gels and bread crumbs, *Cereal Chem.*, 63, 402.
- Zhang, P. et al.(2004). Effects of arabinoxylans on activation of murine macrophages and growth performance of broiler chicks, *Cereal Chem.*, 81, 511.
- Zunft, H.J. et al.(2004). Reduction of postprandial glucose and insulin response in serum of healthy subjects by an arabinoxylans concentrate isolated from wheat starch plant process water, *Asia Pac. J. Clin. Nutr.*, 13 (Suppl.), S147.

CHAPTER II. AIMS

This PhD thesis research project was focused on two whole grains (a modern wheat and an ancient ones - Kamut® khorasan wheat).

Experimental activities were subdivided in two main parts: i) the first aimed to evaluate the combined use of bakery process conditions (including sourdough fermentation and baking temperature) and maturation stage of kernels, to increase the content of bioactive molecules; ii) secondly I focused on the changes of gut microbiota and their metabolites induced by the diet of the two different whole grain foods.

In particular, on the first part I have studied bioactives carbohydrates derived from the hydrolysis or other process induced changes (arabinoxylans, β -glucans, resistant starch, fructans) and polyphenolic components mainly resulting from dough modifications due to the fermentation process (solubilization and acidification) in whole grain cereals (wheat durum and kamut khorasan). During this stage I have evaluated also the effect of kernel stage maturation on the content of these bioactive compounds. The determination of β -glucans, arabinoxylans, resistant starch, fructans have been done according to official methods, and the same for polyphenols.

As whole grain cereals are one of the major source of bioactive compound having many health benefits on human, in the last part II studied the effect of whole grain diet on the gut microbiota and their metabolites. In this part, use an *in vivo* metabolomic approach to evaluate the consumption of whole grain cereal products (kamut korasan vs durum wheat) in 22 human healthy volunteers.

**RESEARCH ARTICLE I. EFFECT OF OF
KERNEL MATURATION STAGE,
SOURDOUGH FERMENTATION AND BAKING
TEMPERATURE ON DIETARY FIBRE AND
PHENOLIC COMPOUNDS**

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Food and Bioprocess Technology, Submitted

Abstract

Several studies have shown that chemical, mechanical, thermal and enzymatic processing can decrease or increase the concentration of dietary fibre and phenolic compounds in whole grain cereal products. This work was aimed to study the influence of maturation stage and bread making process (fermentation and baking) on dietary fibre and phenolic compounds content of whole grain bread.

The amount of the total dietary fibre was mainly influenced by changes accounting to the soluble dietary fibre fraction. Low baking temperature provided in general a high level of fibre which content in fully ripe breads was higher compared to the milky breads, while this last had a higher amounts at high baking temperature.

The sourdough breads showed a high amount of free flavonoids at milky stage compared to other breads. However, they are degraded at high baking temperature while low temperature do not have any effect.

The total polyphenols content was significantly influenced by the maturation stage and fermentation process. In particular, breads obtained by milky stage flour released the highest amounts of total polyphenols in all the fermentation processes considered. On the other hand, sourdough fermentation increased those compounds in bread obtained both with milky and fully ripe seeds.

The impact of the whole food chain on antioxidant compounds may be useful to increase the functional properties of fermented bakery foods. Depending on the food functionality we want to boost, we can use formulation and process to enhance specific bioactive compounds such as dietary fibre or phenolic compound.

1. Introduction

Many scientific studies have been done on health benefits of whole grains cereals due to the fact that they are rich sources of various bioactive compounds such as dietary fibre (arabinoxylans, β -glucans, fructans, lignans), vitamins, minerals and phenolic compounds (Lattimer & Haub, 2010).

Dietary fibre are divided in soluble (SDF) or insoluble (IDF), based on whether they form a solution when mixed with water (soluble), or not (insoluble). The solubility is associated to the structure of polysaccharides; they can be set regularly (insoluble) or irregularly (soluble) on the backbone or as side chains (Mohamed Elleuch et al., 2011). Solubility can be influenced by temperature and ionic strength (Fleury & Lahaye, 1991; Manas et al., 1994) and presence of a substitution group such as COOH or SO₄⁻². The soluble and insoluble nature of dietary fibre involves differences in their technological functionality and physiological effects (Jimenez-Escrig & Sanchez-Muniz, 2000; Roehrig, 1988).

Phenolic compounds are bioactive compounds possessing one or more aromatic rings with one or more hydroxyl groups and generally are classified as phenolic acids, flavonoids, stilbenes, coumarins and tannins (Liu RH, 2004). Phenolic compounds have several protective and physiological functions in human health. They have antioxidant properties and protect against degenerative diseases like heart diseases and cancer in which reactive oxygen species i.e., superoxide anion, hydroxyl radicals and peroxy radicals are involved (Harborne JB, Williams CA., 2000; Rhodes MJ, Price KR., 1997)

Biotechnological processes (such as fermentation and baking) and agronomical practices can increase or decrease the concentration and bioavailability of such compounds on food based cereals. One of the major challenge of the food industry is to find out the better combination between the diverse food processing to increase the concentration of these compounds in food because of their nutritional, and healthy properties for humans.

Several studies have shown that chemical, mechanical, thermal and enzymatic processing can decrease or increase the concentration of dietary fibre and phenolic compounds. The sourdough fermentation can influence the concentration and bioavailability of bioactive compounds found in whole grain cereals (Poutanen et al., 2009). Fermentation of rye bran with yeast was also shown to increase the level of free ferulic acid (Katina et al., 2007a). The antioxidant capacity of traditional rye breads baked with sourdough has been shown to be clearly higher than the common white wheat bread, the highest values reported for breads made with wholemeal flour (Michalska et al., 2007; Martinez-Villaluenga et al., 2009). P. Gelinas and C. M. McKinnon (2006) reported that the concentration of phenolic compounds in wheat varies more according to the farming site than wheat

variety. They also found that baking slightly increases the concentration of phenolics in bread crust specially in white bread than wholemeal bread which already contained high levels of phenolic acids, probably because of Maillard reaction products. Thermal treatments can change the ratio between insoluble–soluble fibre, total dietary fibre content, and their physicochemical properties (Mohamed Elleuch et al, 2011).

Finally the use of immature wheat grain as natural additive has emerged in recent years for improving the health-promoting value of food products. Literature data evidenced that wheat immature wholemeal contains high amounts of antioxidant compounds, due to the presence of considerable quantity of glutathione, ascorbic acid and carotenoids (Paradiso et al., 2006; Humphries and Khachik, 2003). The health benefits of immature wheat grain were demonstrated by both *in vivo* and *in vitro* studies, which showed beneficial effects on the immune response and hypocholesterol activity (Merendino et al., 2006; Paradiso et al., 2006). Moreover, the low levels of gluten proteins and the presence of highly digestible A-type starch granules confer to immature wheat intriguing nutritional properties to be exploited for the development of healthier cereal products (Iametti et al., 2006).

This work was aimed to study the influence of the whole cereal chain (maturation stage and process of durum wheat grain) on the concentration of dietary fibre (insoluble and soluble) and phenolic compounds (polyphenols and flavonoids). For that purpose, the milky and fully ripe of durum wheat matured seed were used to produce sourdough bread baked by two different thermal processes.

2. Materials and methods

Flours

Durum wheat grain (cv. Claudio) was obtained from the Department of Agricultural Sciences, University of Bologna (Italy). Wheat samples were grown at the same location during the same growing season. Grains were collected at the milky (75-79 BBCH scale; 15 days after anthesis) and full ripe maturity stages (89 BBCH scale). Wheat samples were air dried until the 12% humidity was reached and stone milled (100% flour extraction). Both milky and fully ripe stage flour were used for two types of fermentations and baking at two different temperatures.

Fermentation and baking processes

Each flour for the sourdough was mixed with water (45% w/w) and fermented with three strains of lactic acid bacteria (LAB), *L.b plantarum*(98a), *L.b sanfranciscensis*(BB12), *L.b brevis*(3BHI) (Lappi et al. 2010) and one of yeast *Saccharomyces cerevisiae* (LBS) belonging to the collection of

DISTAL and incubated at 30°C for 24 hours. For the industrial fermentation only the *Saccharomyces cerevisiae* strain was used and incubated at 30°C for 1.5 hours.

The fermented dough were baked at two different times and temperatures:

- High time/temperature (250°C for 20 min)
- Low time/temperature (210°C for 10 min)

Analysis of dietary fibres

Soluble and insoluble dietary fibre content was determined according to the procedure developed by Prosky et al. (1988) using the Megazyme assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Samples were enzymatically digested using α -amylase, protease and amyloglucosidase, allowing the determination of IDF and SDF amounts as previously described (Di Silvestro et al., 2012).

Determination of phenolic compounds

The extraction of the free and bound phenolic compounds was performed as previously described (Dinelli et al., 2011). The determination of polyphenol and flavonoid content was carried out according to the spectrophotometric analysis described by Singleton et al. (1999) and Adom and Liu (2002). The gallic acid and catechin calibration curves were used as standard for polyphenol and flavonoid, respectively.

Statistical analysis

Results were calculated from the mean of at least two replicates and expressed in g/100 g of dry matter. Factorial ANOVA was used to evaluate interrelationships between the amount of each dietary fibre components and phenolic compounds of durum wheat based upon the maturation stage (milky and fully ripe), fermentation (sourdough and industrial) and baking (high temperature and low temperature) in breads. These analysis were performed using “R” software, version 3.0.1.

3. Results

Table 1 represent a summary of the content of each phenolic and dietary fibre components quantified in bread samples. In order to discuss the differences induced by the considered variables on the nutraceutical compounds (total, soluble and insoluble forms of fibre, polyphenols and flavonoids), ANOVA analysis was applied to the whole set of data obtained by the different bread samples. In particular, this approach was aimed to describe how the maturation, process variables (fermentation and baking) and their combination were able to influence the amount of bioactive compounds. The results of ANOVA analysis of dietary fibres and phenolic compounds are reported in the supplementary data (Table S1-S3).

As first observation it is clear that free form of every nutraceutical compounds considered was better statistically correlated to the variables considered. It means that the maturation stage, fermentation process, baking process or their interactions were actively involved in the release of free form of bioactive compounds and consequently in the increase of their bioaccessibility.

To better explain the significant interactions of each variable, box plots have been made for dietary fiber, flavonoids and polyphenols.

Table 1. Amount of phenolic compounds and dietary fiber components in analyzed samples

	MW3.HT	MW3.LT	MW4.HT	MW4.LT	RW3.HT	RW3.LT	RW4.HT	RW4.LT
Free polyphenol	85.18 ± 4.48	89.36 ± 5.22	123.31 ± 7.07	162.28 ± 11.72	57.07 ± 5.67	57.63 ± 5.64	118.03 ± 6.76	129.44 ± 5.60
Bound polyphenol	158.11 ± 5.63	147.53 ± 5.70	165.62 ± 3.46	162.28 ± 4.31	136.12 ± 11.60	139.74 ± 11.22	171.19 ± 8.35	153.65 ± 6.94
Total polyphenol	243.29 ± 6.09	236.89 ± 2.78	288.94 ± 10.04	324.57 ± 11.60	193.20 ± 16.63	197.37 ± 15.08	289.22 ± 6.76	283.09 ± 11.18
Free flavonoid	23.25 ± 1.86	22.16 ± 1.82	24.48 ± 0.55	32.48 ± 1.12	24.70 ± 0.69	24.12 ± 2.53	26.23 ± 4.21	23.47 ± 0.49
Bound flavonoid	17.72 ± 1.39	16.85 ± 0.69	18.81 ± 1.48	20.41 ± 1.01	16.27 ± 1.66	17.14 ± 1.07	17.94 ± 2.58	18.31 ± 0.55
Total flavonoid	40.98 ± 3.21	39.01 ± 1.14	43.30 ± 1.95	52.89 ± 1.36	40.98 ± 1.18	41.27 ± 3.47	44.17 ± 6.55	41.78 ± 0.49
Insoluble dietary fiber	13.94 ± 0.27	13.60 ± 0.07	13.03 ± 0.37	11.10 ± 0.19	13.65 ± 0.49	11.61 ± 0.03	11.61 ± 0.007	12.58 ± 0.27
Soluble dietary fiber	0.70 ± 0.17	2.31 ± 0.12	2.78 ± 0.13	1.98 ± 0.16	1.82 ± 0.12	2.40 ± 0.48	1.34 ± 0.07	3.832 ± 0.22
Total dietary fiber	14.65 ± 0.45	15.91 ± 0.19	15.81 ± 0.23	13.08 ± 0.02	15.48 ± 0.36	14.02 ± 0.52	12.96 ± 0.06	16.41 ± 0.50

^a Average values ± s.d., expressed as a percentage of dry matter of enzymatic digestion residue.

MW3.HT: Breads obtained with flour at milky stage using industrial fermentation at high temperature

MW3.LT: Breads obtained with flour at milky stage using industrial fermentation at low temperature

MW4.HT: Breads obtained with flour at milky stage using sourdough fermentation at high temperature

MW4.LT: Breads obtained with flour at milky stage using sourdough fermentation at low temperature

RW3.HT: Breads obtained with flour at fully ripe stage using industrial fermentation at high temperature

RW3.LT: Breads obtained with flour at fully ripe stage using industrial fermentation at low temperature

RW4.HT: Breads obtained with flour at fully ripe stage using sourdough fermentation at high temperature

RW4.LT: Breads obtained with flour at fully ripe stage using sourdough fermentation at low temperature

Dietary fibre

From the ANOVA results it emerged that the interaction (maturation) MAT:TEMP (temperature) was highly significant ($P < 0.01$) for the total and soluble fraction whereas for the insoluble fraction the significant interactions were: MAT:FER (fermentation) and FER:TEMP ($P < 0.01$ and $P < 0.05$ respectively).

Looking at the Fig.1a, it appeared that the maximum amount of IDF was found at the milky stage of the maturation and the industrial fermentation released more of this fibre fraction. The

distribution of the industrially fermented (IND) fully ripe breads and the one of sourdough (SOUR) milky stage were more widespread than their respective counterparts.

Moreover, it resulted that when high temperature is applied (Fig.1b), the industrially fermented breads released more IDF. Independently of the fermentation, we obtained elevated amount of insoluble fibre at high baking temperature.

Fig 1c. illustrates how the temperature influences the soluble fraction considering the maturation stage. The content of SDF is highly correlated to the seed maturation. In fact, as we can observe when the breads are at milky stage higher amount of SDF are released applying high temperature while using low temperature they are at the fully ripe.

The results of the TDF (Fig 1.d) were influenced by the SDF, low baking temperature provided high content in general, and its amount in fully ripe breads was higher at low temperature while milky flour breads accounted for higher amount at high temperature.

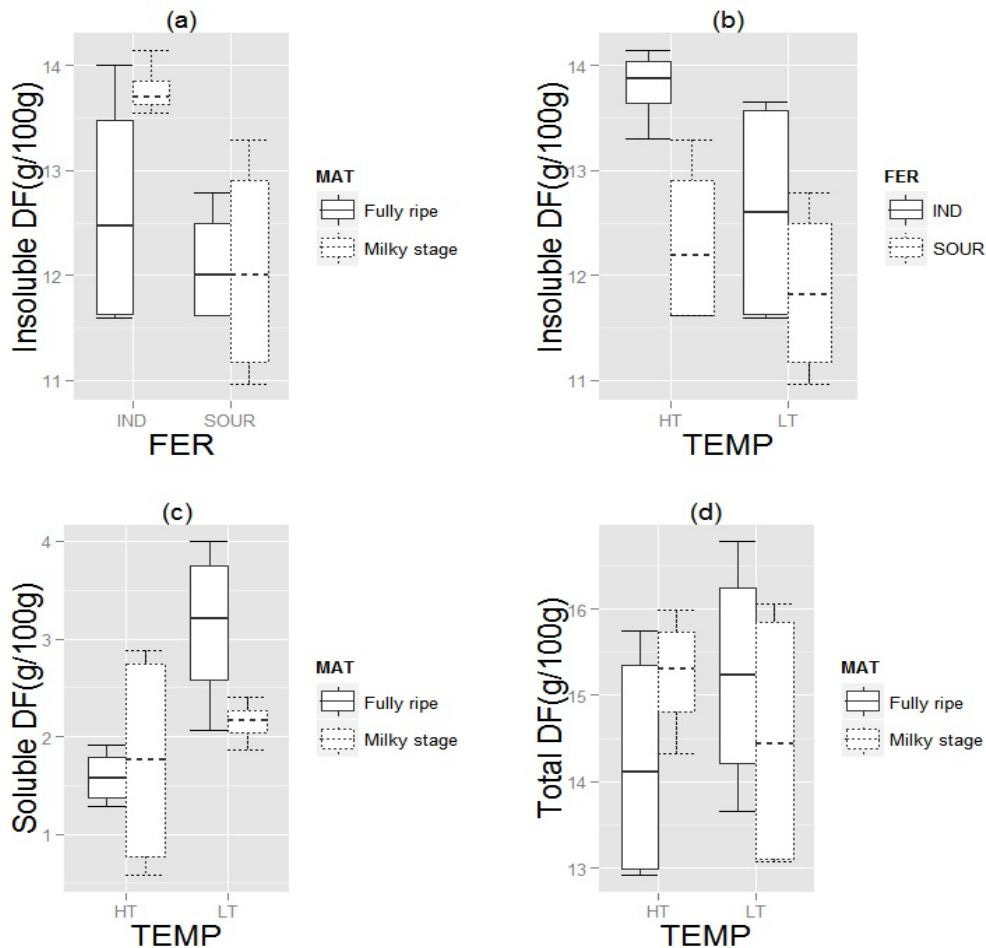


Fig. 1. Significant interactions of IDF content (a) FER:MAT and (b) TEMP:FER and (c).SDF content, TEMP : MAT. (d) on TDF content, TEMP : MAT.

Flavonoids

The interactions MAT:FER and MAT:TEMP resulted highly significant ($P < 0.01$) on the free (FF) and total flavonoids amount (TF) while no interaction was statistically significant for the bound flavonoids (BF).

If the significant interactions of each variable are considered, the sourdough bread showed a high amount of FF at milky stage compared to others (Fig.2). They are degraded at high baking temperature while the low temperature do not have any effect. However at the milky stage the amount increase at high temperature suggesting a different composition or a different interaction with other variables. In general, the sourdough fermentation releases more flavonoids especially at low baking temperature. Even though no interaction was found significant on the BF (Fig.3) the variable fermentation was significant and the sourdough provided a high concentration at milky stage.

The content of the TF was related to the FF content likewise the significant interactions were similar. In fact, the milky stage sourdough increased their content and the low baking temperature do not affect them (Fig.4). The range of the milky stage at low baking temperature is very large compared to other data and at high temperature the maximum level is reached when the IND is applied even though the right-skewness of this data can get it biased.

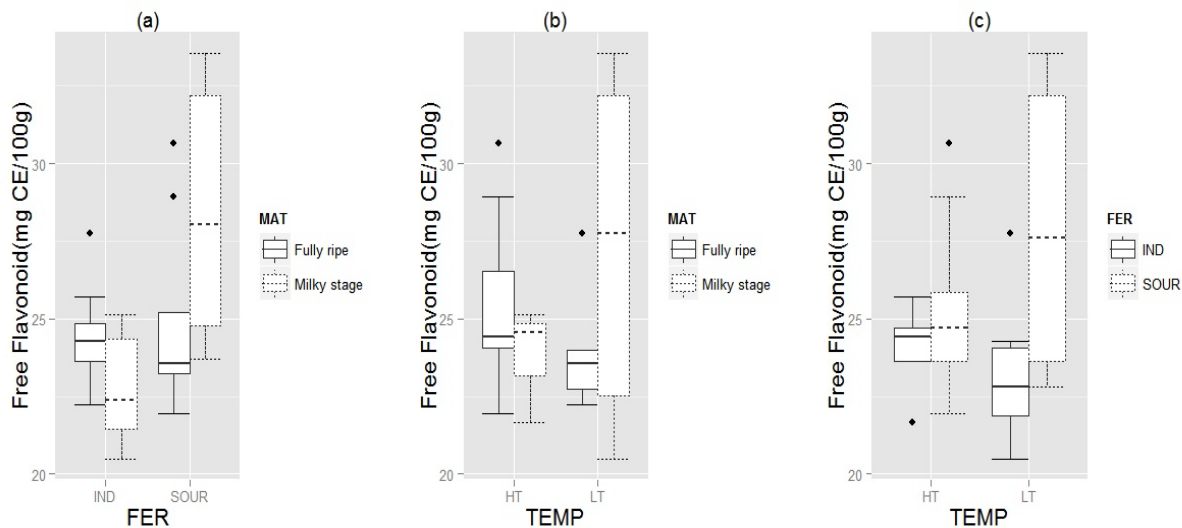


Fig.2. Significant interactions on FF content, FER:MAT, TEMP : MAT and TEMP: FER.

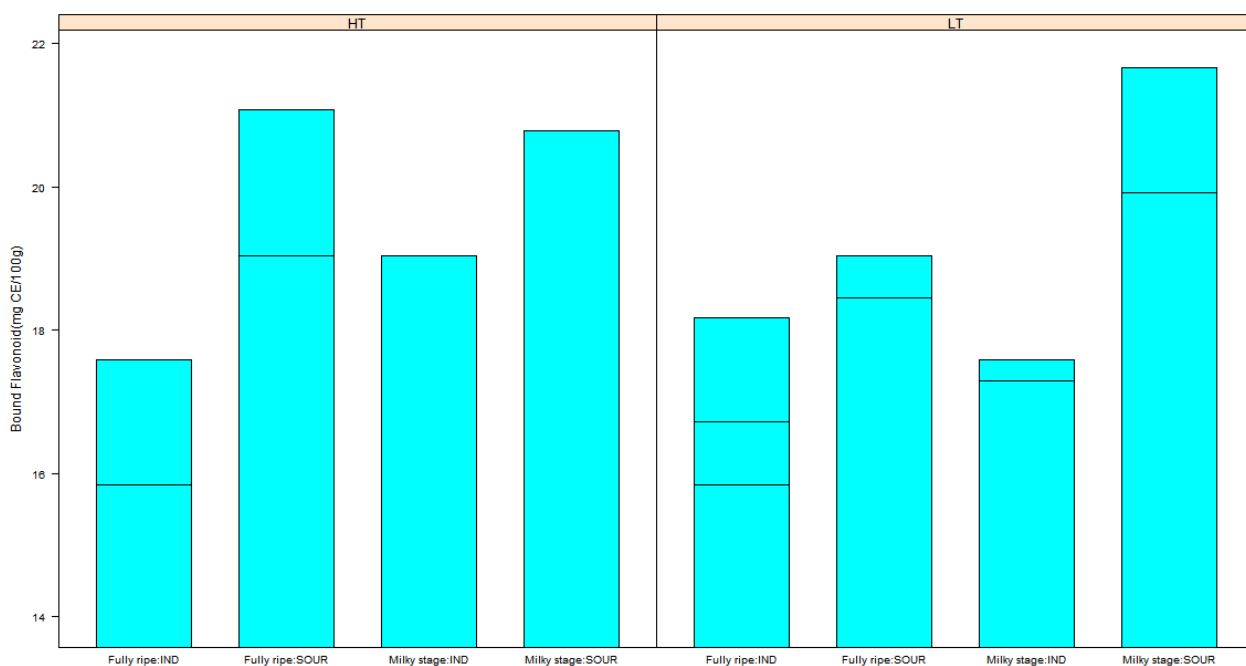


Fig. 3. FB content considering FER:MAT interaction at low and high temperature.

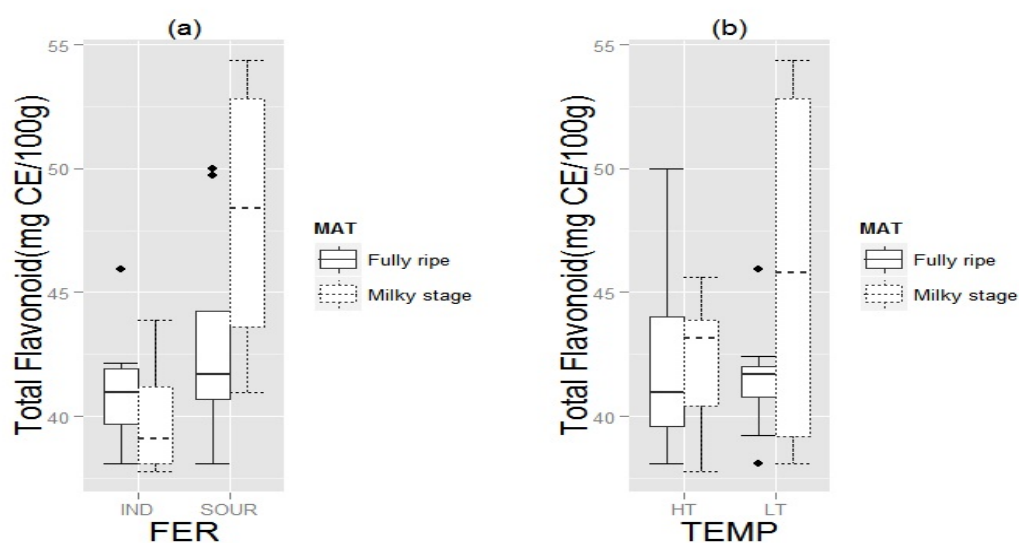


Fig. 4. Significant interactions on TF content, FER:MAT, TEMP:MAT.

Polyphenols

The interactions MAT:FER, MAT:TEMP, and FER:TEMP of free polyphenols (FP) were significant with P-values < 0.05 , < 0.01 and < 0.001 respectively. For the bound (BP) and total polyphenols (TP) only the interaction MAT:FER was significant ($P < 0.05$ and $P < 0.01$ respectively). The FP were linearly influenced by each variable considered and by the combination

of those characterizing the bakery process (fermentation and baking) as resulted by their high statistical significance.

In particular sourdough fermentation, milky stage and with a lesser extent, low baking temperature influenced the release of FP (Fig. 5.a). The breads obtained by the combination of milky stage flour sourdough fermented and baked at low baking temperature reached the highest content of FP (Fig. 5.a-c). Moreover, the sourdough fermentation positively affected the concentration of FP strongly greater than the other variables considered in this study.

Concerning BP all the single variables and the interaction MAT:FER were statistically significant. In fact the maturation stage and baking temperature cycle showed a null or not uniform trend (Fig. 5.d). Finally, TP were significantly influenced by the maturation stage and fermentation process (Fig 5.e). In particular milky stage released the highest amounts of TP in all the fermentation processes considered. On the other hand, sourdough fermentation increased those compounds in bread obtained both by milky and fully ripe seeds.



Fig. 5. Significant interactions of FP content (a) FER:MAT, (b) TEMP:MAT and (c) TEMP: FER., (d). BP content, FER:MAT, (e). TP content, FER:MAT.

As insoluble dietary fibre consists of lignin, cellulose and some hemicelluloses that do not form gels due to their water insolubility and which fermentation is very limited in the human gastrointestinal tract (Lattimer & Haub, 2010), it is conceivable to explain the high amount of IDF found as effect of industrial fermentation because yeast cannot degrade them. In general the content of dietary component is high at the milky stage as described by De Gara et al.(2003) confirming our outcomes in breads samples. In general DF content decreases during dough mixing and fermentation (Hansen et al., 2002; Lambo, Öste, & Nyman, 2005). Many of the observed changes in dietary fibre degradation (Boskov Hansen et al., 2002) or solubilisation (Katina et al., 2007a), can be explained by the contribution of endogenous enzymes, especially xylanases. On the other hand, starch gelatinization and retrogradation together with the formation of protein polysaccharide complex through Maillard reaction could be responsible for the increase of IDF as well as the oxidation of phenolic compounds (Vitagione et al., 2008).

Hansen et al. (2002) have shown that dough mixing, proofing and baking had a minor effect on total phenolic content in wholemeal rye bread. May be this could explained the slight differences we found in industrially fermented bread specially in the total flavonoids contents. Specifically, during fermentation, because of the consumption of oxygen by yeast, further oxidation due to oxidative enzymes is prevented and thus changes in the levels of phytochemical compounds are minimal during this stage (Leenhardt et al., 2006; Hidalgo et al., 2010; Hansen et al., 2002; Vogrincic et al., 2010; Hidalgo & Brandolini, 2010). In particular, Angioloni and Collar (2010) found a decrease in phenol content due to oxidation in cereals and pseudocereals after mixing. They observed that the phenol content was higher in the flour than the respective breads and illustrated how enzymes such oxygenase and peroxidase (which are present in flour) are activated by the addition of water during mixing, and possibly promote the decrease of phenol content. The retention of total phenolic compounds occurred during sourdough fermentation, was described by Gianotti et al. (2011) in Kamut[®] khorasan wheat bread. The amount of total phenolic compounds after baking was similar to that detected in the correspondent Kamut[®] khorasan flour. These results suggested that sourdough fermentation might counteract the thermal loss of phenolic compounds due to baking process. In fact as reported by (Katina et al., 2007), sourdough fermentation increased the level of total extractable phenols and of free ferulic acid.

The resulted degradation of phenolic at high temperature during sourdough is in accordance with Friedman (2004) who considered that baking destroyed much phenolic compounds to explain the different results obtained in DF. The effect of baking process on bioactive compound found in literature is not always clear.

In fact, while Gelinas & McKinnon (2006) showed an increase of phenolic compound, Leenhardt et al. (2006) reported that phenolic compounds are destroyed during baking process.

Vitali et al. (2009) studied the effect of apple fibre, oat fibre, soya flour, amaranth, and carob on the nutritional characteristics of biscuits. The authors reported that the biscuit baking process did not affect total phenol content as calculated from the sum of soluble and insoluble phenols. However, a significant raise was found in the levels of the soluble phenols and a considerable decrease in insoluble phenols in all samples following baking. This outcome was attributed to the high temperature during baking which could destroy some of the complexes of insoluble phenols with other food components. Thus, the reduction of the total amount of flavonoids and polyphenols that we observed in milky breads fermented with sourdough at high temperature can be explained by the results obtained by Vitali et al. (2009).

4. Conclusion

In conclusion fermentation and baking process influenced the functional properties of bread by leading to a reformulation of some bioactive compounds. Lactic acid bacteria and yeast fermentation may increase or retain the content of bioactive compounds present in flour. In particular, sourdough increases the content of phenolic compounds while industrial fermentation increases the fibre components. In general low temperature retained more bioactive compounds when the grain was at fully ripe stage whereas, independently of the baking process more of these compounds were extracted at the milky stage of the grain. Our findings may be useful to understand the effect of the whole food chain on antioxidant compounds and thereafter to increase the functional properties of fermented bakery foods.

Depending on the food functionality we want to boost, we can modulate formulation and process to enhance specific bioactive compounds such as dietary fibre or phenolic compounds. This work provides a new approach in the innovation strategies along the whole chain of bakery functional foods.

References

- Adom KK, Liu RH (2002). Antioxidant activity of grains. *J Agric Food Chem* 50: 6182-6187.
- Boskov Hansen, H., Andersen, M.F., Nielsen, L.M., Bach Knudsen, K.-E., Meyer, A.S., Christensen, L.P., Hansen, A. (2002). Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread making. *Eur. Food Res. Technol.* 214, 33–42.
- Di Silvestro, R., Marotti, I., Bosi, S., Bregola, V., Segura-Carretero, A., Sedej, I., Mandic, A., Sakac,

- M., Benedettelli, S., Dinelli, G., (2012). Health-promoting phytochemicals of Italian common wheat varieties grown under low-input agricultural management. *Journal of the Science of Food and Agriculture* 92, 2800-2810.
- Dinelli, G., Segura-Carretero, A., Di Silvestro, R., Marotti, I., Fu, S., Benedettelli, S., Ghiselli, L., Fernandez-Gutierrez, A., (2011). Profiles of phenolic compounds in modern and old common wheat varieties determined by liquid chromatography coupled with time-of-flight mass spectrometry. *Journal of Chromatography A* 1218, 7670-7681.
- Fleury, N., & Lahaye, M. (1991). Chemical and physico-chemical characterisation of fibres from *Lamiaria digitata* (Kombu Breton): A physiological approach. *Journal of the Science of Food and Agriculture*, 55, 389–400.
- Friedman, M. (2004) Effects of food processing. In: *Encyclopedia of Grain Science*, Vol. 2 (edited by C. Wrigley, H. Corke & C.E. Walker). Pp. 328–340. Kidlington, Oxford, UK: Elsevier Academic Press.
- Gélinas P, McKinnon C M. (2006). Effect of wheat variety, farming site, and bread-baking on total phenolics. *Int. J. Food Sci. Technol.* 41: 329-332
- Hansen, H. B., Andreasen, M. F., Nielsen, M. M., Larsen, L. M., Bach Knudsen, K. B., Meyer, A. S., et al. (2002). Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. *European Food Research and Technology*, 214, 33–42.
- Harborne JB, Williams CA. (2000). Advances in flavonoid research since 1992. *Phytochemistry* 55: 481-504.
- Humphries, J.M., Khachik, F., (2003). Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. *Journal of Agricultural and Food chemistry* 51, 1322-1327.
- James M. Lattimer and Mark D. Haub.(2010). Effects of Dietary Fiber and Its Components on Metabolic Health. *Nutrients* 2, 1266-1289.
- Jimenez-Escrig, A. and Sanchez-Muniz, F. J. (2000). Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research*, 20, 585–598.
- K. Poutanen, Laura Flander, Kati Katina. (2009). Sourdough and cereal fermentation in a nutritional perspective *Food Microbiology* 26 693–699.
- Katina, K., Laitila, A., Juvonen, R., Liukkonen, K.-H., Kariluoto, S., Piironen, V., Landberg, R., Aman, P., Poutanen, K., (2007a). Bran fermentation as a means to enhance technological properties and bioactivity of rye. *Food Microbiol.* 24, 175–186.

- Iametti, S., Bonomi, F., Pagani, M.A., Zardi, M., Cecchini, C., D'Egidio M.G., (2006). Properties of the protein and carbohydrate fractions in immature wheat kernels. *J. Agric. Food Chem.* 54, 10239–10244.
- Lambo, A. M., Öste, R., & Nyman, E. M. G.-L. (2005). Dietary fibre in fermented oat and barley β -glucan rich concentrates. *Food Chemistry*, 89, 283–293.
- Laura De Gara, Maria C. de Pinto, Vita M. C. Moliterni, and Maria G. D'Egidio.(2003). Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *Journal of Experimental Botany*, Vol. 54, No. 381, pp. 249–258
- Leenhardt, F., Lyan, B., Rock, E. et al. (2006). Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *Journal of Agricultural and Food Chemistry*, 54, 1710–1715
- Liu RH. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 134: 3479S–3485S.
- Manas, E., Bravo & Saura-Calixto, F. (1994). Source of error in dietary fiber analysis. *Food Chemistry*, 50, 331–342.
- Martinez-Villaluenga, C., Michalska, A., Frias, F., Piskula, M.-K., Vidal-Valverde, C., Zielinski, H., (2009). Effect of flour extraction rate and baking on thiamine and riboflavin content and antioxidant capacity of traditional rye bread. *J. Food Sci.* 74, 49–55.
- Merendino, N., D'Aquino, M., Molinari, R., De Gara, L., D'Egidio M. G., Paradiso, A., Cecchini, C., Corradini, C. Tomassi, G., (2006). Chemical characterization and biological effects of immature durum wheat in rats. *J. Cereal Sci.* 43, 129 –136.
- Michalska, A., Ceglinska, A., Amarowicz, R., Piskula, M.-K., Szawara-Nowak, D., Zielinski, H., (2007). Antioxidant contents and antioxidative properties of traditional rye breads. *J. Agric. Food Chem.* 55, 734–740.
- Mohamed Elleuch, Dorothea Bedigian, Olivier Roiseux, Souhail Besbes, Christophe Blecker, Hamadi Attia (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry* 124, 411–421
- Paradiso, A., Cecchini, C., De Gara, L., D'Egidio, M. G., (2006). Functional, antioxidant and rheological properties of meal from immature durum wheat. *Journal of Cereal Science* 43, 216–222.
- Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W., Furda, I., (1988). Determination of insoluble, soluble, and total dietary fibre in foods and food products. Interlaboratory study.

Journal of the Association of Official Analytical Chemists 71, 1017-1023.

Rhodes MJ, Price KR (1997). Identification and analysis of plant phenolic antioxidants. Eur J Cancer Prev 6: 518-521.

Roehrig, K. L. (1988). The physiological effects of dietary fiber. Food Hydrocolloids, 2, 1–18.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology 299, 152–178.

Vitaglione P., Napolitano A., Foliano V. (2008). Cereal dietary fiber, a natural functional ingredient to deliver phenolic compounds in the gut. Trends Food Sci Technol 19: 451-461.

**RESEARCH ARTICLE II. METABOLOMIC
ANALYSIS OF SOURDOUGH FERMENTED
DOUGH AND BREAD OBTAINED BY
DIFFERENT IMMATURE WHEAT FLOURS**

Danielle Taneyo Saa & Andrea Gianotti
Food Chemistry, Submitted

Abstract

The aim of this study was to investigate the metabolite profiling of sourdough obtained by *L.b plantarum* 98a, *L.b sanfranciscensis* BB12, *L.b brevis* 3BHI and *Saccharomyces cerevisiae* LBS and bakery products made from immature grain using gas chromatography mass spectrometry and electronic nose.

Electronic nose sensors did not distinguish between the single variables (cereal genotypes, maturation stage and fermentation) but a combination of them may be observed.

The pool of volatile compounds of the Kamut® khorasan dough was mainly characterized by the release of alcohols such as 1-hexanol, 1-pentanol, ketones (3-hydroxy-2-butanone, 2-octanone) and carboxylic acids whereas the durum wheat was characterized by D-limonene, 2,5-dimethylfuran, and aldehydes such as hexanal, decanal.

This metabolomic approach can represent a valuable instrument to predict the release of volatile compounds and the flavouring impact of a flour based upon the fermentation process. As well as to rapidly determine the traceability and authenticity of fermented cereals products.

1. Introduction

The taste and aroma are very important attributes for the food industry, especially in bakery and pastry industry. The different composition of immature grain may represent a good alternative to modulate either for their dietary fibre content or to improve the flavour of cereal products (Shewry et al., 2011; Skogerson et al., 2010)

The fermentation and baking process can influence the flavour of fermented foods in different ways. During a lactic acid fermentation, usually the most evident change is the production of acid and lowering pH leading to an increase in sourness. Given that most of the acid produced during fermentation will be produced by the metabolism of sugars, sweetness will probably decrease as sourness increases (Mcfeeters., 2004). It has been demonstrated that sourdough microflora mainly lactobacilli and yeasts, have important metabolic interactions contributing to a production of flavour compounds (De Vuyst & Heysens, 2005). The production of volatile compounds in sourdough is influenced by the lactic acid bacteria (LAB) activity and the sourdough yeasts. Generally, LAB are responsible of lowering pH. Both LAB and yeasts are able to release aroma precursors, such as free amino acids, and it has been reported that the concentrations of free amino acids increased significantly during sourdough fermentation (Hansen et al. 1989a).

Prolonged dough fermentation may produce intense proteolysis and higher amounts of free amino acids that can operate as precursors of Strecker aldehydes mostly responsible for 'malty' odor note, such as methylpropanal, 2- and 3-methylbutanal, and methional (Zehentbauer & Grosch, 1998b). For example, long fermentation time increases the concentration of 3-methyl-butanol and 2-phenyl-ethanol (Hansen & Hansen, 1996). These compounds are directly related to the fermentative activity of the yeast in the dough fermentation. Also acetic acid concentration is higher in bread made with long fermentation time. It has been reported that the primary role of yeast in sourdough is leavening whereas LAB release important flavour (Salim et al., 2006).

A study reported that evaluation of fruit maturation and shelf-life consisting of sensing the aromatic volatiles emitted can be done using electronic olfactory systems (Benady, 1995). Metabolic modifications are mostly imputable to: post-harvest ripening, respiration, fermentation and phenolic oxidation (Young et al., 1996).

Analysis of VOCs (volatiles compound) by using olfactometry system in cereals and their products is being applied with increasing occurrence in cereal food technology laboratories. Even though, most of these analysis focus on mycological safety issues, they can be a key tool to improve the sensory attributes of cereal products. Metabolic profiling requires the analysis of metabolites groups

associated either with a specific metabolic pathway or comprising a class of compounds (Dettmer et al., 2007). The study of metabolite profiling in fermented foods is applied to observe metabolite modifications during fermentation and the possibility to predict, among others, the sensory and nutritional quality of the fermented final product (Mozzi et al., 2013).

The aim of this study was to investigate the metabolite profiling of cereals products made from immature grain by using gas chromatography mass spectrometry and electronic nose during a sourdough fermentation.

2. Material and Methods

2.1. Flours

Kamut[®] khorasan grain and durum wheat grain (cv. Claudio) were obtained from the Department of Agricultural Sciences, University of Bologna (Italy). Wheat samples were grown at the same location during the same growing season. Grains were collected at the milky (75-79 BBCH scale; 15 days after anthesis) and full ripe maturity stages (89 BBCH scale). Wheat samples were air dried until the 12% humidity was reached and stone milled (100% flour extraction).

2.2. Fermentation and baking processes

Each flour for the sourdough was mixed with water (45% w/w) and fermented with three strains of LAB, *L.b plantarum*(98a), *L.b sanfranciscensis*(BB12), *L.b brevis*(3BHI) and one of yeast *Saccharomyces cerevisiae* (LBS) belonging to the collection of DISTAL and incubated at 30°C for 24 hours. For the industrial fermentation only the *Saccharomyces cerevisiae* strain was used and incubated at 30°C for 1.5 hours.

The dough were baked at two different baking cycles (time/temperatures):

- High time/temperature (250°C for 20 min)
- Low time/temperature (210°C for 10 min)

Table 1. Abbreviations of the samples names

Wheat genotype (GEN)	Durum wheat (W) Kamut (K)	
Maturation stage (MAT)	Milky stage (M) Fully ripe(R)	
Fermentation (FER)	SOUR IN	Sourdough Industrial (straigh process)

2.3. *Solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) analysis*

A polydimethylsiloxane-divinylbenzene-carboxen coated fibre (DVB-carboxen/PDMS, 50/30 μm) and a manual SPME holder (Supelco Inc., Bellefonte, PA, USA) were used after preconditioning according to the manufacturer's instruction manual. Before each head space sampling, the fibre was exposed to the GC inlet for 10 min for thermal desorption at 250°C in a blank sample. Five grams of dough or bread were placed in 10 mL vials following sealed. The samples were then equilibrated for 10 min at 50°C. The SPME fibre was exposed to each sample for 40 min and finally the fibre was inserted into the injection port of the GC for a 10 min sample desorption. The temperature program was: 50 °C for 0 min, then programmed at 1.5 °C/min to 65 °C and finally at 3.5 °C/min to 220 °C which was maintained for 20 min. Injector, interface and ion source temperatures were 250, 250 and 230 °C, respectively. Injections were carried out in splitless mode and helium (3 mL/min) was used as carrier gas.

All GC-MS raw files were converted to netCDF format via Chemstation (Agilent Technologies, Palo Alto, CA, USA) and subsequently processed with the XCMS toolbox (<http://metlin.scripps.edu/download/>) for automatic and simultaneous retention time alignment, matched filtration, peak detection, and peak matching. The resulting table containing information such as peak index (retention time-m/z pair) and normalized peak area was exported into R 3.0.0 (www.r-project.org) for subsequent statistical analysis (Serrazanetti et al., 2011).

Identification of molecules was carried out by comparing their retention times with those of pure compounds (Sigma-Aldrich, Milan, Italy), and confirmed by searching mass spectra in the available databases (NIST version 2005 and Wiley version 1996) and literature. Quantitative data of the identified compounds were obtained by interpolation of the relative area versus the internal standard area (Serrazanetti et al., 2011).

2.4. *Electronic nose*

An electronic nose device PEN2, provided by (WMA Airsense Analysentechnik GmbH) Schwerin, Germany, was used. The portable electronic nose PEN2 has an array of 10 different metal oxide sensors (Table 1 describes the properties of sensors used) positioned into small chamber ($V = 1.8$ mL). Seven grams of dough were placed in 10 ml vials and the vials sealed. The headspace inside the vials was equilibrated for 20 min. The headspace gas was pumped over the sensors of the electronic-nose with a flow of 400 mL/min. During the measurement phase, the bomb pushes the volatiles through a closed loop that includes the measurement and concentration chambers. No air enters or exits the loop. The measurement phase lasts 120 s, time enough for sensors to reach a

stable value. The collected data interval was 1 s. The sampling temperature was 25°C and injection temperature was 180°C.

2.5. Statistical analysis

Canonical analysis of principal coordinates (CAP) have been performed in the R environment using the vegan package (Oksanen et al., 2013) to discriminate between and within the dataset based on metabolomic data (Di cagno et al., 2011). The results of this analysis give two different plots represented on the canonical axis 1: the first, called the “CAP score plot”, illustrates a summary of the relationship among the observations (or samples), and the second plot “loading score plot” shows a summary of the variables (properties) that gives an idea of which molecules discriminate more between the different group of the score plot. The value of the score plot will be negative or positive on the canonical axis 1 helping to identify specified molecules, positive and/or negative in both groups of loadings plot.

3. Results

3.1. Electronic Nose

A CAP analysis has been done for the dough samples analyzed with e-nose, in Fig. 1a-1d are reported the loading plot and score plot of the canonical axis 1 which gave the major percentage of explained variation (76.73%). The above results of electronic nose (Fig. 1a-1d) showed that the sensors were not able to separate between the two different types of fermentation or maturation stage of the kernel as well as between Kamut® khorasan and durum wheat genotype. In Fig. 1a, we observed a general trend, along the negative pole of axe 1 they are Kamut® khorasan samples: fully ripe Kamut® khorasan dough fermented with LAB (RK-SOUR) and milky Kamut® khorasan dough fermented industrially (MK-IND) while on the line (± 0) they are the durum wheat fully ripe counterparts (RW-IND and RW-SOUR). Even though there is not a clear separation along axe 1, we can separate these samples between positive and negative values. Looking at the negative and positive values, we plotted the loading coefficient (Fig.1b) or sensors that are discriminating in this dataset. At the negative pole, the sensors mainly responsible of the discrimination are S3 (aromatic), S5 (aromatic-aliphatic), S1 (aromatic), S7 (sulphur-organic) whereas at the positive pole we have S2 (broad-range), S10 (methane-aliphatic), S4 (hydrogen). On the other hand the sensors that have less influence, are S9 (sulphur-chlor) and S6 (broad-methane) at the positive axe and S8 (broad-alcohol) at the negative.

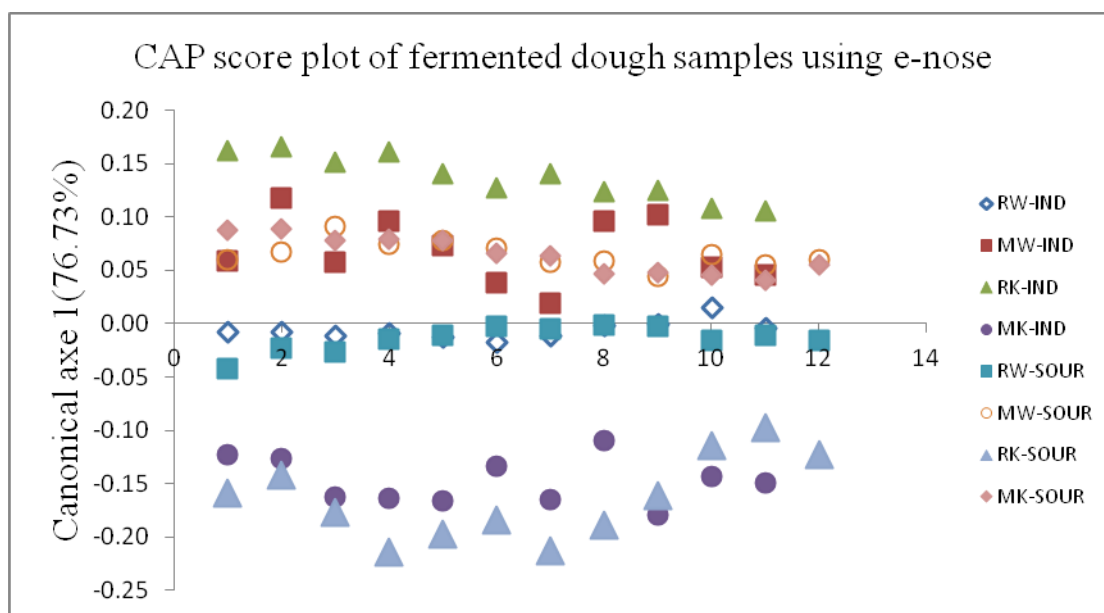


Fig. 1a. CAP score Plot of Kamut vs. Wheat dough samples using E-nose;
RW-IND /SOUR: wheat fully ripe IND/SOUR, MW-IND/SOUR: wheat milky stage IND/SOUR,
RK-IND/SOUR: kamut fully ripe IND/SOUR, MK-IND/SOUR: kamut milky stage.

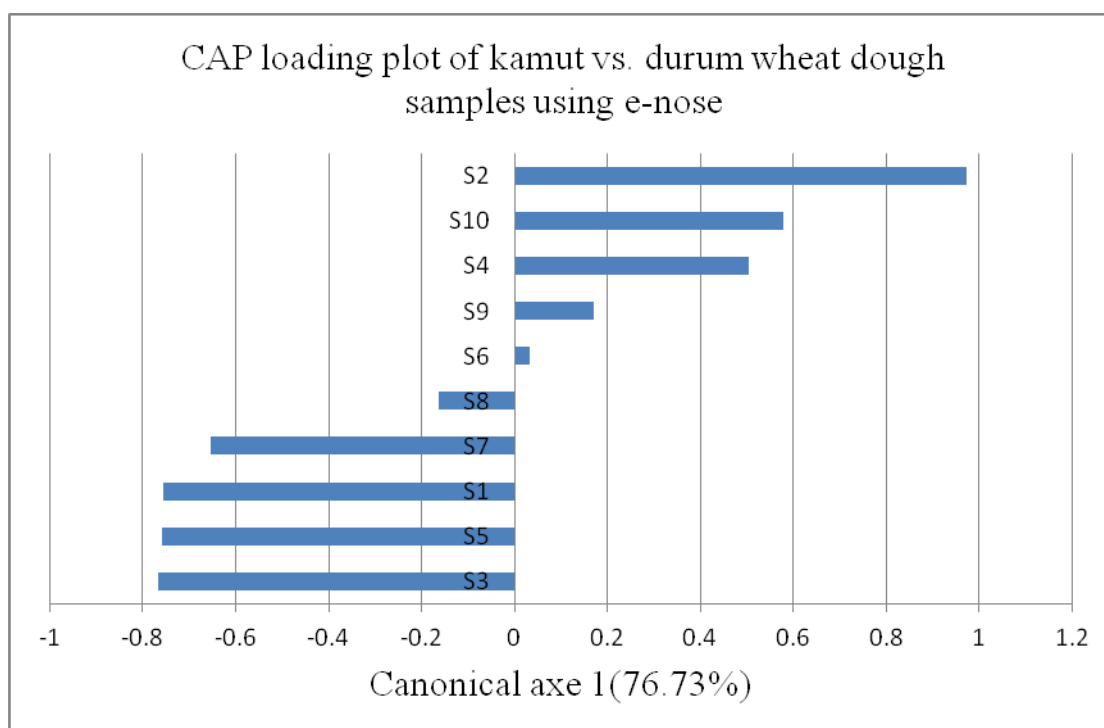


Fig. 1b. CAP loading Plot of Kamut vs. Wheat dough samples using E-nose

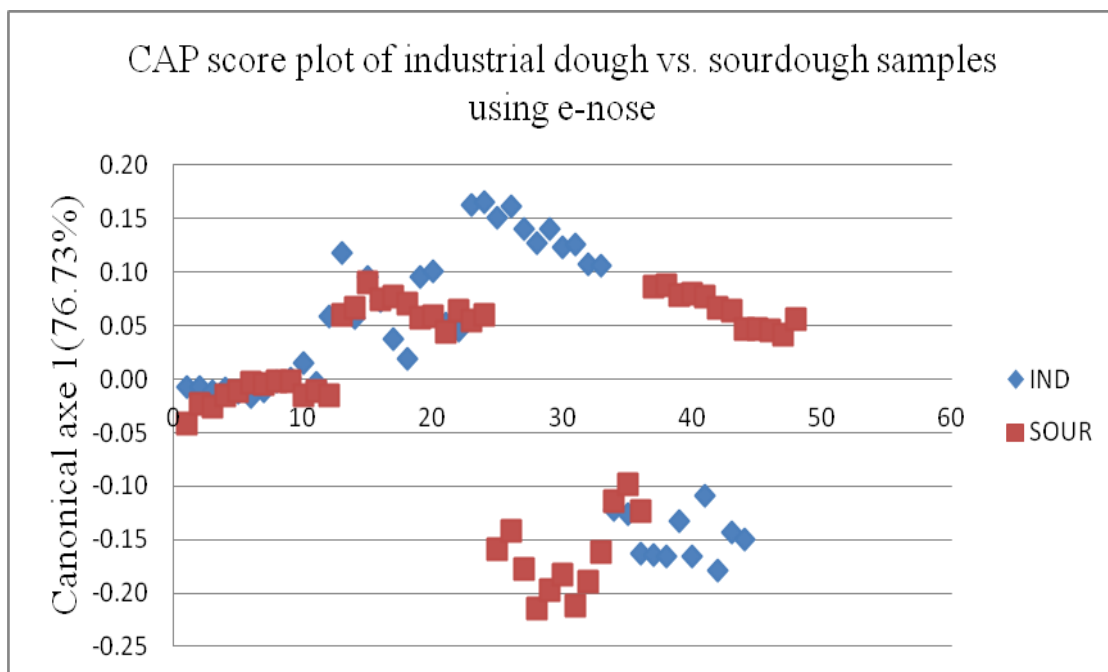


Fig. 1c. CAP score plot of Industrial vs. Sourdough dough samples using E-nose

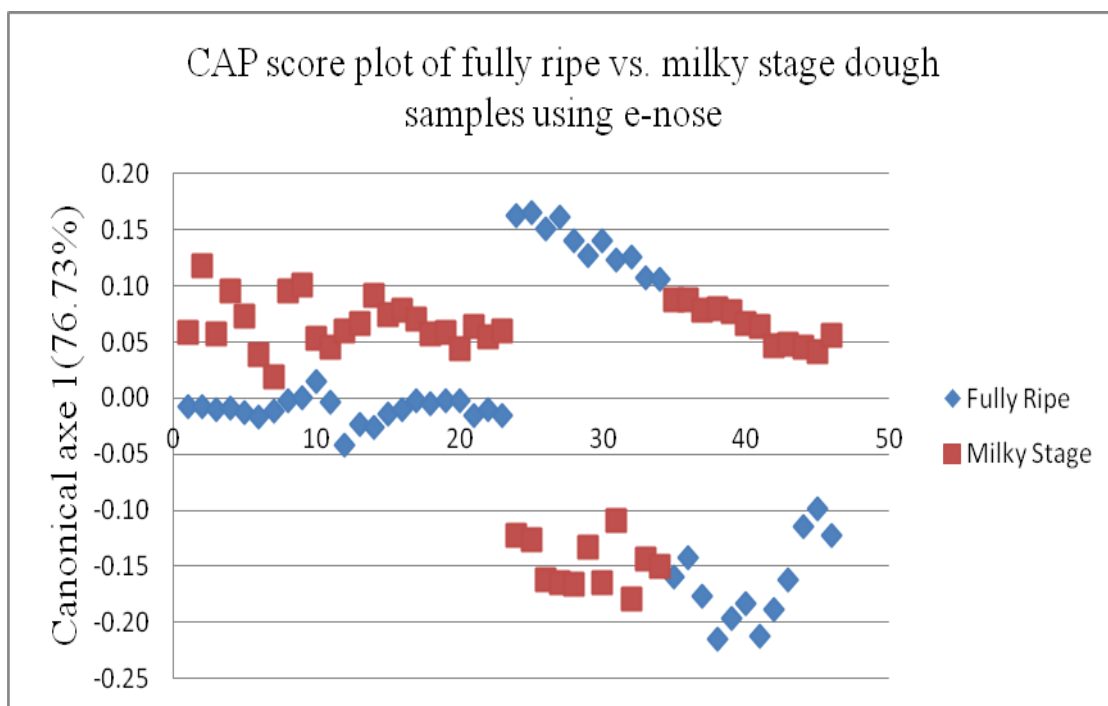


Fig. 1d. CAP score Plot of fully ripe vs. milky stage dough samples using E-nose

3.2. Solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) analysis

Up to 75 molecules belonging to the families of alcohols, aldehydes, ketones, carboxylic acids, esters, phenols, organic acids, and hydrocarbons were detected in dough and bread samples. In

Fig.2 is represented the canonical axis 1 which explained 57.20% of the total variation of the dough samples dataset.

In general the pool of volatile compounds appeared to be different between Kamut® khorasan and durum wheat dough samples independently of the fermentation and maturation stage. The pool of Kamut® khorasan dough's volatile compounds is along the negative pole of axis 1 as showed in Fig.2 and the molecules released were mainly alcohols (1-hexanol, 1-pentanol, Z-4-dodecenol, 2-hexanol, 3-methyl-1-butanol), 2-butyl-2-octanal, ketones (3-hydroxy-2-butanone, 2-octanone, 4-methyl-2-hexanone), and carboxylic acids (3-methyl pentanoic acid, p-tert-butyl benzoic acid, 3-nonynoic acid methyl ester, acetic acid and pentanoic acid).

Concerning durum wheat which molecules are along the positive pole of axis 1 (Fig. 2), we have mainly D-limonene, 2,5-dimethylfuran, aldehydes (hexanal, decanal, acetaldehyde and (E)-2-octenal), aromatic hydrocarbon compounds (ethylbenzene, p-xylene, 1-ethyl-1-methylcyclohexane), and some hydrocarbons compounds (3-ethyl-2-methyl-1,3-hexadiene, 1-chlorooctadecane). It appears that the Kamut® khorasan dataset had a broadly range and more volatile compounds compared to durum wheat.

The total amount of volatile compounds quantified in baked samples was less than the fermented ones. As we can observe on Fig.3a, the CAP analysis did not allow a good separation of baked samples either based on maturation stage or on the baking conditions, but taking into account the variable "fermentation" we obtained a very good separation (Fig.3b). As we can examine on Fig.3b industrial baked breads are well separated from sourdough baked breads along the canonical axis 1 which explained 51.30% of the total variation. Fig. 4 shows the volatile compounds that discriminated the baked fermented samples along the axis 1. Ethyl alcohol, 3-hydroxy-2-butanone, acetic acid, octanoic acid, heptanoic acid, 3-methyl butanoic acid are the compounds principally responsible for the discrimination of sourdough breads whereas heptanal, acetophenone, ethylbenzene, 2-methyl-3-decen-5-one, 2,2,5-trimethylhexane, 2,4-bis(1-methylethyl)phenol, are those responsible of industrial fermentation discrimination.

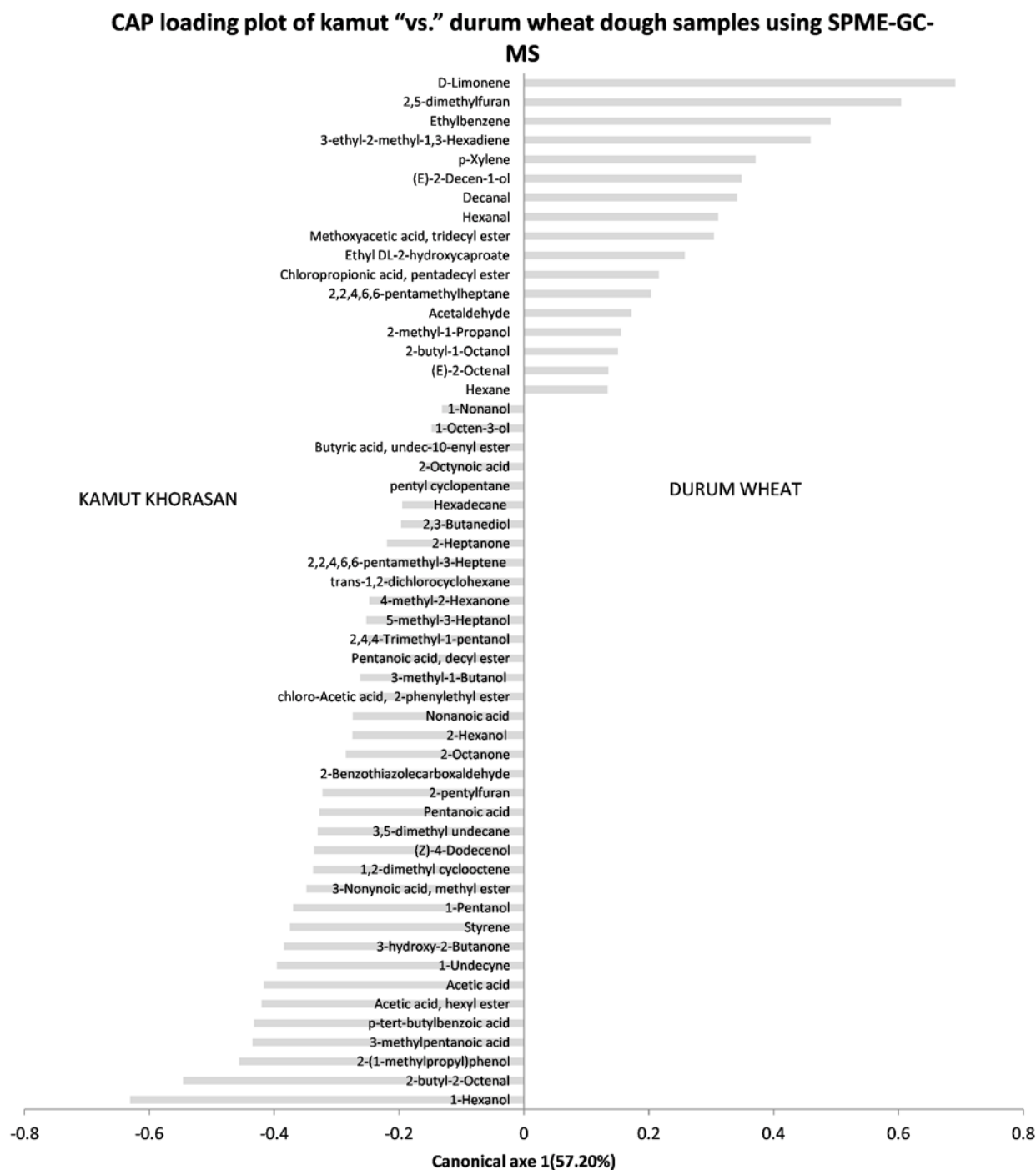


Fig. 2. CAP loading Plot of Kamut vs. Wheat dough samples using E-nose

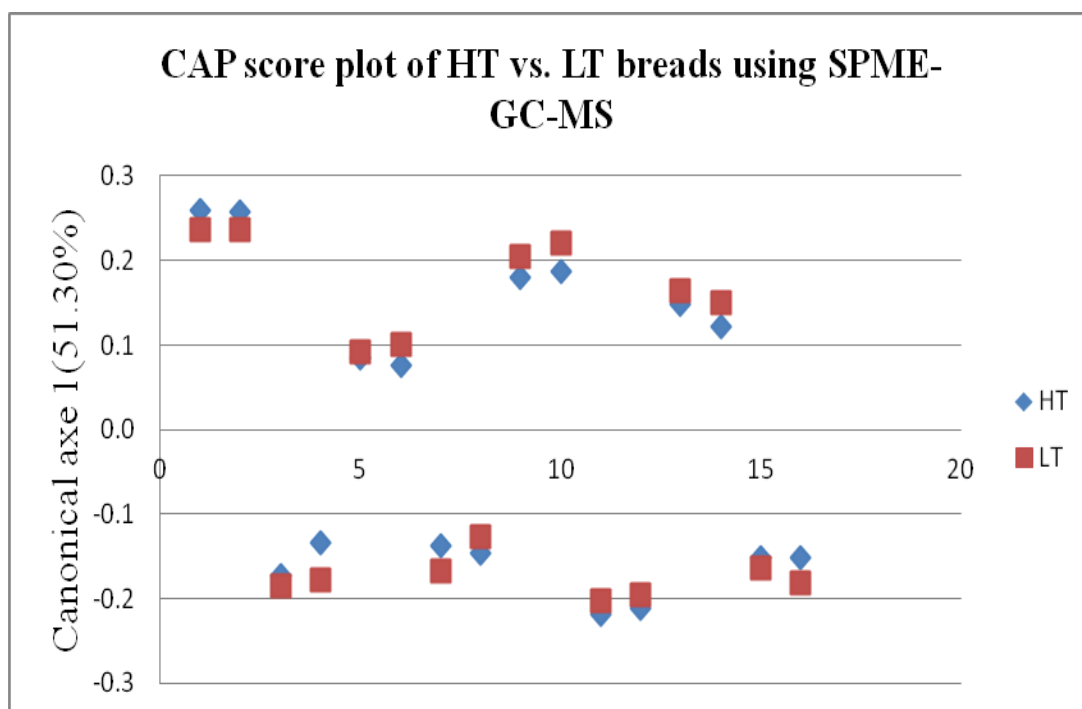


Fig. 3a. CAP score Plot of HT vs. LT breads using GC

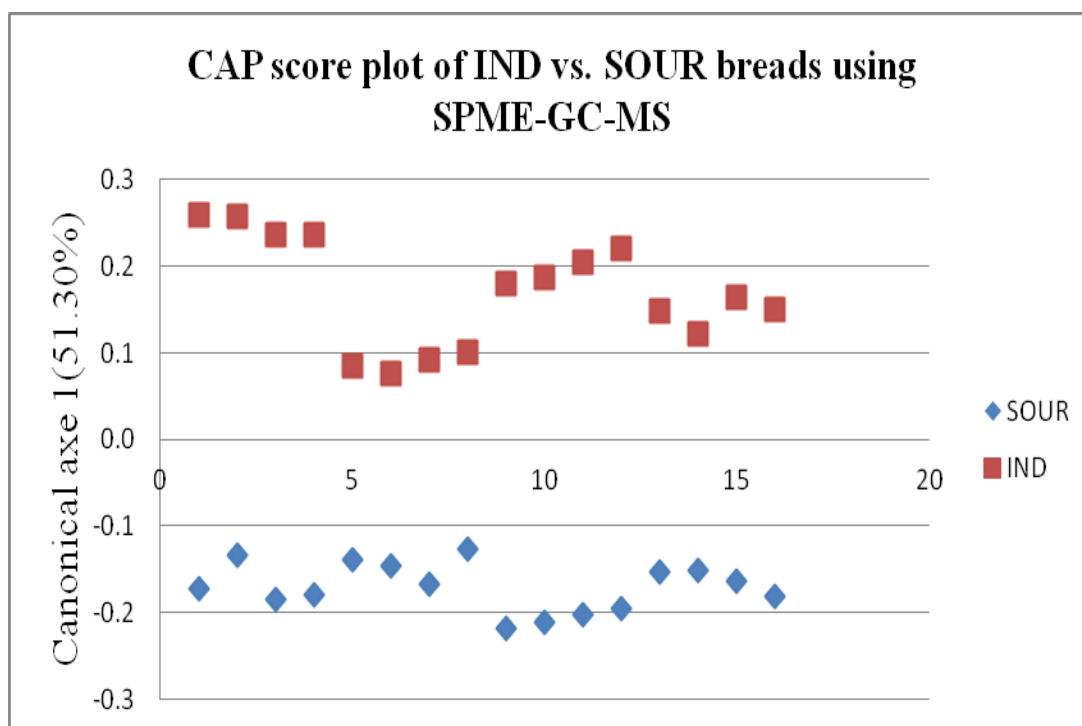


Fig. 3b. CAP score Plot of industrial fermentation vs. sourdough breads using GC

CAP loading plot of kamut “vs.” wheat breads using SPME-GC-MS



Fig. 3c. CAP loading Plot of IND vs. SOUR breads using GC

4. Discussion

In this study, it emerges that the electronic nose sensors cannot distinguish the different cereal genotypes, stage of kernel development and fermentation but we observed a trend due to some combinations of these variables. In fact, within the Kamut® khorasan group the fully ripe sourdough samples (RK-SOUR) were almost symmetric along the axis 1 (in opposite direction) to the fully ripe industrially fermented dough (RK-IND). Similarly the milky industrially fermented samples (MK-IND) were almost in the opposite direction to the milky sourdough samples (MK-SOUR). This could be explained by the different groups of compounds existing in the raw flour. Some authors reported that the grain composition can differ according to the stage of kernel development and to the specific types of processing applied (Shewry et al., 2011; Skogerson et al., 2010). Actually, as we expected, different flours produce different volatile profiling accordingly to their role as substrate for the LAB fermentation. Kamut® has more protein than wheat, leading to more differentiation of sulphuric compounds (S9). Even though several studies reported positive applications of electronic nose technology to the discrimination of fruit of different qualities, with oranges (Gomez, et al., 2006), tomatoes (Berna, et al. 2004), apples (Young et al., 1999; Brezmes et al., 2000; Brezmes et al., 2001; Saevels et al., 2003) and cherry (Toivonen et al, 2006), but till now few literatures refer to cereals if not only for a mycology aspect. The sensors used for the electronic nose were probably inappropriate for our samples leading to minor discrimination of these.

We observed that the pool of volatile compounds of the Kamut® khorasan samples, identified using the SPME-GC-MS, constituted a more complex metabolite profiling (more compounds at the negative pole which belong to the Kamut® khorasan samples), as well as sourdough had more aromatic compounds than industrial dough. It is known that the primary metabolic products of LAB are lactic acids and acetic acids responsible for the sensory qualities of sourdough bread with citric and malic acids that are also produced by LAB but in lesser amounts (Gamze Yazar & Sebnem Tavman, 2012; Kam et al., 2011). Therefore the production of acetic acid as one of the major discriminant acid in Kamut® khorasan maybe ensued to the interactions between flour nutrients and naturally occurring microflora or sourdough LAB.

The presence of ethanol we have found in sourdough is also in agreement with others studies (Hansen & Hansen, 1994, Vernocchi et al., 2008). These authors demonstrated that ethanol and ethyl acetate were generated in the highest amounts in sourdough fermented by *Lb. sanfranciscensis*, while ethyl-n-propanoate, butyl-acetate and n-pentyl acetate were only produced in sourdoughs started with yeasts. The effect of the microbial activity on the volatile compound synthesis have been well studied (Meignen et al., 2001, Gobbetti & Corsetti, 1997). Schieberle

(1996) showed that alcohols can be produced by yeast via the Ehrlich pathway by transamination of some amino acids into the corresponding α -keto acids, followed by a decarboxylation into the aldehyde and finally reduction into the alcohol, explaining the detection of aldehydes such as heptanal, hexanal, 3-methyl-2-butanol in industrial fermentation. Likewise the production of hexanal and decanal in wheat is due to amino acid degradation.

Some metabolites have been detected at high concentrations in durum wheat grain and which significantly distinguished this cereal from the others, included butyrolactone, alpha-pinene, propylbenzene, 1-ethyl-3-methylbenzene, cymene and limonene (Beleggia et al. 2009; Maciej et al., 2010). This authors confirm our findings as D-limonene and ethyl benzene highly discriminated durum wheat fermented samples. Even the presence of benzene derivatives and terpenes has been found significantly higher in wheat durum as previously identified by others authors (Maciej et al., 2010, Jelen & Wasowicz, 1998). Lee et al. (2013) concluded that processing can impacted the metabolic activity of individual grains and grain fractions using a non-targeted metabolomic approach confirming the different distributions we obtained according to the stage of kernel development or the fermentation/baking process used.

5. Conclusion

Our findings evidenced the contribution of sourdough process to enhance the differences among the raw flours. The potential use of flour from immature grain as an ingredient in bakery can be valuable either for their dietary fibre content or to improve the flavouring of cereals products. The application of olfactometry system such as e-nose can be useful but it is important to consider the sensors specificity which have to be suitable for that food matrix. Using metabolomic approach, we can predict the release of volatile compounds and the flavouring impact of a flour based upon the fermentation process. Finally, this approach represents a promising tool to rapidly determine as far as possible the traceability and authenticity of fermented cereals products.

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References

- Beleggia, R., Platani C., Spano, G., Monteleone, M., Cattivelli, L. (2009). Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. *J Cereal Sci*, 49, 301–309.

- Benady M. (1995). Fruit ripeness determination by electronic sensing of aromatic volatiles. *Transactions of the ASAE*, 38, 251-257.
- Berna, A. Z., Jeroen, L., Stijn, S., Corrado, D. N., & Nicolai, M. B.(2004). Electronic nose systems to study shelf life and cultivar effect on tomato aroma profile. *Sensors and Actuators B*, 97, 324-333.
- Brezmes, J., Llobet, E., Vilanova, X., Orts, J., Saiz, G., & Correig, X.(2001). Correlation between electronic nose signals and fruit quality indicators on shelf-life measurements with pink lady apples. *Sensors and Actuators B*, 80, 41–50.
- Brezmes, J., Llobet, E., Vilanova, X., Saiz, G., & Correig, X. (2000). Fruit ripeness monitoring using an Electronic Nose. *Sensors and Actuators B*, 69, 223–229.
- De Vuyst, L. & Heysens, P. (2005). The sourdough microflora: Biodiversity and metabolic interactions. *Trends in Food Science & Technology*, 16, 43-56.
- Dettmer K, Pavel A, Aronov PA, Hammock BD. (2007). Mass spectrometry-based metabolomics. *Mass Spectrom Rev*, 26, 51–78.
- Di Cagno, R., De Angelis, M., De Pasquale, I., Ndagijimana, M., Vernocchi, P., Ricciuti, P., Gagliardi, F., Laghi, L., Crecchio, C., Guerzoni, M. E., Gobbetti, M., & Francavilla, R. (2011). Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization. *BMC Microbiology*, 11, 219.
- Gamze Yazar & Sebnem Tavman.(2012). Functional and Technological Aspects of Sourdough Fermentation with *Lactobacillus sanfranciscensis*. *Food Eng Rev*, 4, 171–190
- Gobbetti, M., Corsetti, A. (1997). *Lactobacillus sanfrancisco* a key sourdough lactic acid bacterium: a review. *Food Microbiol*, 14, 175–187
- Gomez, A. H., Wang, J., Hu, G. X., & Pereira, A. G. (2006). Electronic nose technique potential monitoring mandarin maturity. *Sensors and Actuators B*, 113, 347–353.
- Hansen, A. & Hansen, B. (1994). Volatile compounds in wheat sourdoughs produced by lactic acid bacteria and sourdough yeasts. *Z Lebensm Unters Forsch*, 198, 202–209
- Hansen, A. & Hansen, B. (1996). Flavour of sourdough wheat bread crumb. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, 202, 244–249.
- Hansen, A., Lund, B. & Lewis, M.J. (1989a). Flavour of sourdough rye bread crumb. *Lebensmittel Wissenschaft und Technologie*, 22, 141-144.
- Jelen, H & Wasowicz, E. (1998). Volatile fungal metabolites and their relation to the spoilage of agricultural commodities. *Food Rev Int*, 14, 391-426.
- Kam, W.Y., Wan Aida, W.M., Sahilah, A.M. & Maskat, M.Y. (2011). Volatile Compounds and

- Lactic Acid Bacteria in Spontaneous Fermented Sourdough. *Sains Malaysiana*, 40, 135–138
- Lee, DP., Alexander, D., Jonnalagadda, SS. (2013). Diversity of Nutrient Content in Grains - A Pilot Metabolomics Analysis. *J Nutr Food Sci*, 3, 191
- Maciej Busko, Henryk Jelen, Tomasz Goral, Jaroslaw Chmielewski, Kinga Stuper, Lidia Szwajkowska-Michalek, Bozena Tyrakowskad and Juliusz Perkowski. (2010). Volatile metabolites in various cereal grains. *Food Additives and Contaminants*, 27, 1574-1581
- Mcfeeters. R.F. (2004). Fermentation Microorganisms and Flavor Changes in Fermented Foods Fermentation Technology-12th World Congress of Food Science and Technology.
- Meignen B, Onno B, Gelinas P, Infantes M, Guilois S, Cahagnier B .(2001). Optimization of sourdough fermentation with *Lactobacillus brevis* and baker's yeast. *Food Microbiol*, 18, 239-245.
- Mozzi, F., Ortiz M.E., Bleckwedel, J., De Vuyst L., Pescuma M. (2013). Metabolomics as a tool for the comprehensive understanding of fermented and functional foods with lactic acid bacteria. *Food Research International*, 54, 1152-1161
- Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). *vegan: Community Ecology Package*. R package version 2.0-7. <http://CRAN.R-project.org/package=vegan>.
- Saevels, S., Lammertyn, J., Berna, A. Z., Veraverbeke, E. A., Di Natale, C., & Nicolai, B. M. (2003). Electronic nose as a non-destructive tool to evaluate the optimal harvest date of apples. *Postharvest Biology and Technology*, 30, 3–14.
- Salim-ur-Rehman, Paterson, A. & Piggott, J.R. (2006). Flavour in sourdough breads: A review. *Trends in Food Science & Technology*, 17, 557-566.
- Schieberle, P. (1996). Intense aroma compounds – useful tools to monitor the influence of processing and storage on bread aroma. *Advances in Food Science (CMTL)*, 18, 237-244.
- Serrazanetti, D. I., Ndagijimana, M., Sado, S. L., Corsetti, A., Vogel, R. F., Ehrmann, M., & Guerzoni, M. E. (2011). Acid stress-mediated metabolic shift in *Lactobacillus sanfranciscensis* LSCE1. *Applied and Environmental Microbiology*, 77, 2656-2666.
- Shewry PR, Van Schaik F, Ravel C, Charmet G, Rakszegi M, et al. (2011). Genotype and environment effects on the contents of vitamins b1, b2, b3, and b6 in wheat grain. *J Agric Food Chem*, 59, 10564-10571.
- Skogerson K, Harrigan GG, Reynolds TL, Halls SC, Ruebelt M, et al. (2010). Impact of genetics and environment on the metabolite composition of maize grain. *J Agric Food Chem*, 58, 3600-3610.
- Toivonen, P.M. A., Kappel, F., Stan, S., McKenzie, D. L., & Hocking, R. (2006). Factors affecting

the quality of a novel fresh-cut sweet cherry product. *LWT—Food Science and Technology*, 39, 240–246.

Vernocchi P., Ndagijimana M., Serrazanetti D., Gianotti A., Vallicelli M., Guerzoni M.E. (2008).

Influence of starch addition and dough microstructure on fermentation aroma production by yeasts and lactobacilli. *Food Chem*, 108, 1217–1225

Young, H., Gilbert, J. M., Murria, S. H., & Ball, R. D. (1996). Causal effects of aroma compounds on royal gala apple flavors. *Journal of the Science of Food and Agriculture*, 71, 329–336.

Young, H., Rossiter, K., Wang, M., & Miller, M. (1999). Characterization of Royal Gala apple aroma using electronic nose technology potential maturity indicator. *Journal of Agriculture and Food Chemistry*, 47, 5173–5177.

Zehentbauer, G., & Grosch, W. (1998b). Crust aroma of baguettes. II. Dependence of the concentrations of key odorants on yeast level and dough processing. *Journal of Cereal Science*, 28(1), 93–96.

**CHAPTER III. INFLUENCE OF KERNEL
MATURATION STAGE AND BREAD
MAKING ON THE CONTENT OF
RESISTANT STARCH, FRUCTANS AND β -
GLUCANS**

1. Introduction

β -glucans, fructans and resistant starch are part of dietary fiber component that we can find in cereals whole grain. β -glucans are the non-starch polysaccharides and components of the cell wall of cereals starchy endosperm, fructans are mainly found in aleurone layer whereas resistant starch is the starch fraction that is not absorbed, nor digested in the human small intestine (Cummings and Englyst 1991). The health benefits of cereals whole grain, or precisely of their dietary components it is not longer to be claimed. In fact, the use of whole grain flour is increased for the production of healthy breads as the consumption of whole grain has been demonstrated to reduce the risk of colorectal cancer, cardiovascular diseases, diabetes and obesity (Slavin, 2004; Topping, 2007) and also to improve technological properties of bread (Holtekjolen et al., 2008). The effect of oat and barley β -glucans in reducing the risk of coronary heart disease (CHD) has been recognized by the U.S. Food and Drug Administration (FDA), and now the EFSA approves two health claims on both oat-and barley-based foods. Wheat fructans are not digested in the small intestine (Nilsson and Björck 1988; Nilsson et al 1988), so they are considered part of traditional dietary fiber (DF) and provide substrates for microbiota. There has been much attention in the prebiotic and bifidogenic properties of fructans and, especially, the inulin-type fructans. They have prebiotic effects, because they selectively stimulate the growth and/or activity of one or a limited number of micro-organisms in the gut microbiota that confers health benefits to the host (Roberfroid et al. 2010). Several studies involving dietary incorporation reported that fructans increase gastrointestinal bifidobacteria (Meyer and Stasse-Wolthuis, 2009) and mineral absorption (Scholz-Ahrens et al. 2007), and reduce appetite (Cani et al. 2009). Resistant starch in food, exist in four subtypes: RS1, RS2, RS3, RS4, each with its characteristics and properties. Likewise the NSP (non-starch polysaccharides), they are involved in promoting large bowel health and preventing bowel inflammatory diseases (IBD) and colorectal cancer (CRC) (Topping, Anthony, & Bird, 2003).

In general, it is observed that wheat yield and functional properties of the derived wheat flour may substantially change as a result of genetic factors and agronomic factors. Agronomic factors such as temperature, water availability, solar radiation and soil fertility have a great impact on the developing grain kernels during kernel filling and strongly determine cereals products quantity and quality (E. Dornez et al., 2008). Thus, the accumulation of dietary component on the kernel may also vary. Another factor which can be considered for the accumulation of dietary component is the kernel stage maturation or grain development (Sully Philippe et al., 2006). While the variability in grain quantity is a major interest for farmers and, thus, quite a lot of mathematical models relating grain yield to environmental conditions have been developed (Jamieson et al., 1998; Landau et al.,

2000). The variables that affect the grain quality are one of the major challenges for the milling and processing industries.

Some studies demonstrated that technological processes such as cooking, baking and fermentation have an influence on the β -glucans, fructans and resistant starch content. As an example, Johansson et al. (2007) evaluated the effect of processing on β -glucans using three hydrolysis methods: acid hydrolysis (AH) with H₂SO₄, lichenase hydrolysis (LH) and the modified AOAC 995.15 method (MH). They observed that cooking increase the amount of soluble β -glucans whereas baking decreased it. Moreover Fermentation have been shown to reduces RS content (Abd-Elmoneim et al.,2004) while baking increases it.

Therefore, food industries are strongly interested in founding better technological processes to increase the daily dietary fiber intake of people, without a direct incorporation of cereals whole grains into food. In this study we evaluated the impact of seeds maturation, fermentation and baking processes on the content of β -glucans, fructans and resistant starch on breads.

2. Materials and methods

2.1 Flours

Kamut flour and wheat flour, was obtained from DISTA. The seed stage maturation of each was milky and fully ripe, and each flour was used for two types of fermentations and baking at two different temperatures.

2.2 Fermentation and baking processes

Each flour for the sourdough was mixed with water (45% w/w) and fermented with three strains of lactic acid bacteria (LAB), *L.b plantarum*(98a), *L.b sanfranciscensis*(BB12), *L.b brevis*(3BHI) (Lappi et al. 2010) and one of yeast *Saccharomyces cerevisiae* (LBS) belonging to the collection of DISTAL and incubated at 30°C for 24 hours. for the industrial fermentation only the *Saccharomyces cerevisiae* strain was used and incubated at 30°C for 1.5 hours.

The fermented dough were baked at two different times and temperatures:

- High time/temperature (250°C for 20 min)
- Low time/temperature (210°C for 10 min)

2.3 Determination of β -glucan, resistant starch and fructans

All the samples were analyzed at least in duplicate. An essay kit of mixed-linkage of β -glucans (Megazyme International Ireland Ltd) was used for the determination β -glucan according to McCleary and Holmes (1985), McCleary and Codd (1991), McCleary and Mugford (1992) and AOAC 2002.02. The samples were treated with a solution of ethanol(50%), sodium phosphate

buffer (pH 6.5, 20mM) was added and then incubated in a boiling water bath. The tubes were equilibrated at 50 °C, treated with lichenase enzyme (50U/mL ; Megazyme International Ireland Ltd) and then further incubated for 60 min at 50 °C. Sodium acetate buffer (pH 4.0, 50mM) was added into the tubes and then centrifuged for 10min, after which aliquots were removed and treated with β -glucosidase (2 U/ml, Megazyme International Ireland Ltd) for another 10min. The reaction mixture was incubated with glucose oxidase peroxidase reagent (GOPOD; Megazyme International Ireland Ltd) for 20min and the absorbance read at 510nm against a reagent blank.

Resistant starch (RS) was determined according to AACC 32-40 method and McCleary and Monaghan (2002). 100 mg of sample were weighed and sodium maleate buffer (pH=6.0, 100mM) containing pancreatic amylase and amyloglucosidase (3 U/mL, Megazyme International Ireland Ltd) added in. Samples were carefully mixed and incubated for 16 h at 37 °C under stirring conditions. The tubes were then treated with 50% ethanol, and centrifuged at 1500 g for 10min. The supernatants were decanted, the pellets re-suspended in 50% ethanol, and the process repeated twice.

The pellets from centrifugation were treated with potassium hydroxide (2 M) in an ice bath under stirring for 20min. Sodium acetate buffer (pH=3.8, 100mM) , and then amyloglucosidase (3300 U/mL, Megazyme International Ireland Ltd) were added and the tubes incubated for 30 min at 50 °C. Aliquots were removed and treated with GOPOD reagent (Megazyme International Ireland Ltd). These were incubated at 50 °C for 20min and the absorbance read at 510nm, against a reagent blank. Glucose solution (1 mg/mL) was used as a standard.

Fructan was extracted with hot water (80°C) and quantification was performed according to McCleary, Murphy, and Mugford (2000) using a spectrophotometric method with the enzymatic assay kit K-FRUC (Megazyme International Ireland Ltd). 1,000 g of samples were homogenized in 80mL of distilled water on a magnetic stirrer at 80°C for 20 min. The solutions were cooled to room temperature and the volume adjusted to 100mL, and . then centrifuged at 1500g for 15 min. Then, 200 mL of the supernatant was incubated with 200 mL sucrase (100 units)/maltase (1,000 units) mixture at 40°C for 30 min. Then, 500mL of 100mM sodium acetate buffer (pH 4.5) was added and 200 mL aliquots of the resulting digest placed into two spectrophotometer cuvettes. To one of these, 100 mL of 100mM sodium acetate buffer (pH 4.5) was added (Solution A) and to the other 100 mL fructanase (exo-inulinase, endo-inulinase)was added (Solution B), and both were incubated at 40°C for 20 min. To each cuvette, 2mL distilled water, 200 mL imidazole buffer (2M, pH 7.6) and 100 mL NADPb(150mg)/ATP (440 mg) mixture was added. After 3 min (T1), absorbances were read at 340 nm on a spectrophotometer, then, 20 ml enzyme mixture containing hexokinase (425 units/

mL)/phosphoglucose isomerase (840 units/mL)/glucose 6-phosphate dehydrogenase (212 units/mL) was added and the absorbances were measured at 5- min intervals until reaction completion (T2). The fructan content was calculated by the difference in the change in absorbance between Solution A (sucrase, maltase) and Solution B (sucrose, maltase, fructanase). The determinations were made after each mixing.

Table 2. Abbreviations of the samples names

Wheat genotype (GEN)	Durum wheat Kamut	
Maturation stage (MAT)	Milky stage Fully ripe	
Fermentation (FER)	SOUR IN	Sourdough Industrial (straigh process)
Baking process	HT	High time/temperature
	LT	Low time/temperature

2.4 Statistical analysis

Results were calculated from the mean of at least two replicates and expressed in g/100 g dry matter. Factorial ANOVA was applied to determine interrelationships between the amount of each resistant starch, β -Glucans and fructan content based upon the flour genotype(kamut and durum Wheat), maturation stage(milk and fully ripe), fermentation (sourdough and industrial) and baking (high temperature and low temperature) in breads. These analysis were performed using “R” software, version 3.0.1.

3. Results

From the results of the four-way ANOVA with interaction, it appeared that the interaction genotype-maturation (GEN:MAT) and genotype-fermentation (GEN:FER) was highly significant with a p-value respectively $p < 0.01$ and $p < 0.001$ for the β -glucans. Box plots of each significant results have been done. Likewise the box plots of resistant starch were made only for the significant interaction: genotype-fermentation (GEN:FER) ($p < 0.01$), and maturation-fermentation (MAT:FER) ($p < 0.001$). The same approach was used for fructans data and the interaction genotype-fermentation and maturation-fermentation resulted highly significant ($p < 0.001$). The median amount of β -glucans (BG) found in durum wheat was greater than the amount of kamut, independently of the maturation

stage and the fermentation. The interaction of the stage maturation and the genotype (FIG 1.a) shows that the maximum amount attained in bread samples of milky stage was greater than the fully ripe, but the median of milky stage was greater only for kamut probably because the overall range of the data set is higher for durum wheat milky stage. This difference in median may also due to the left-skewness of durum wheat fully ripe data set. The genotype-fermentation interaction of breads, indicated that the median content of industrial fermentation was higher than the sourdough fermented breads (FIG 1.b). The interquartile range of each batch of data resulted different, as durum wheat sourdough resulted to be widely distributed whereas the industrial counterpart was right-skewed with one outlier.

The fig 2, shows the amount of beta-glucans on baked bread taking into account the interaction maturation –fermentation. The content of β -glucans was higher in durum wheat in all the combination except for the milky stage-sourdough combination whereas the differences were weak. It emerged that with the high temperature/long time, the highest value is observed on durum wheat fully ripe industrially fermented, while for low temperature/ short time is the milky stage counterpart which showed the highest value.

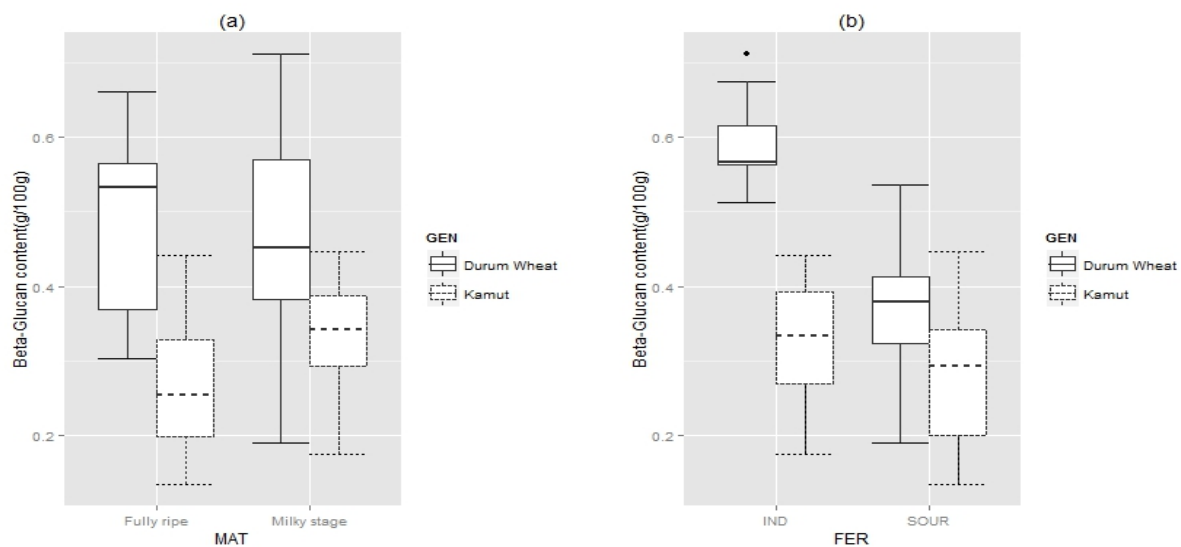


Fig.1. Amount of Beta-glucan on genotype flour (GEN) Kamut and Durum wheat based on the stage of maturation (MAT)(a) and the type of fermentation(FER)(b).

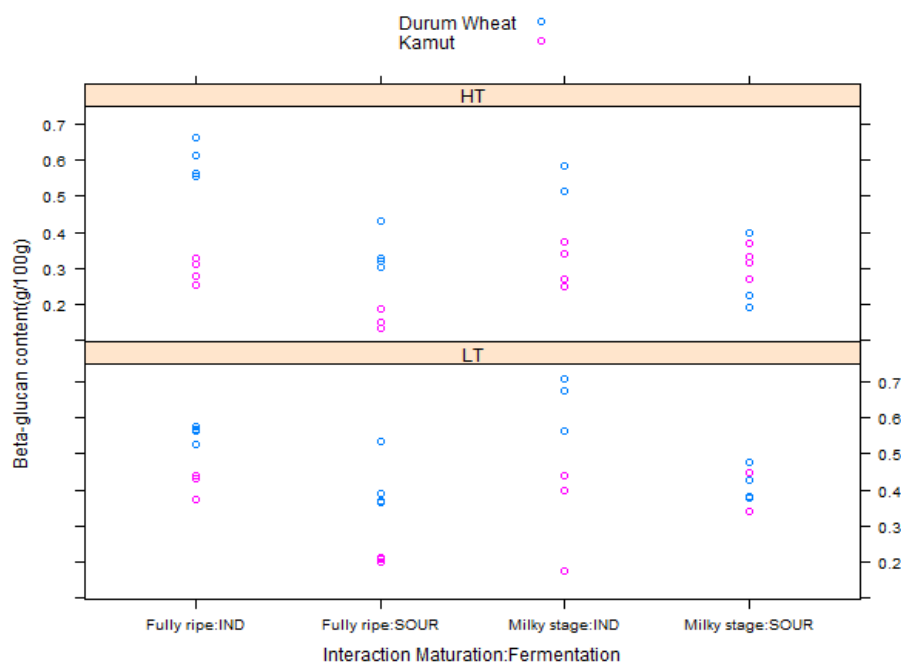


Fig.2. Amount of β -glucans on baked breads considering the maturation-fermentation interaction.

Looking at the resistant starch data set (FIG.3), it seemed that durum wheat had a better value than kamut independently of the fermentation type, as well as the fully ripe amount was greater than milky stage. In the interaction GEN-FER (FIG.3.a), we observed that the median amount of resistant starch was higher in the industrial fermentation while the median of durum wheat industrially fermented was lower than kamut counterpart. In general, these data were widely distributed and right skew causing the variations in the median data. The MAT-FER (FIG.3.b) interaction illustrates that the median of the resistant starch of fully ripe is greater than milky stage, and the industrial fermentation median is higher than sourdough. The data of the fully ripe at industrial fermentation appeared to have a wide range and its counterpart seems to be slightly right-skew.

In these baked products, the durum wheat at fully ripe industrial fermentation showed the maximum amount of resistant starch independently of the temperature applied (FIG.4). Similarly, the sourdough breads had a lower amount compared to the industrial fermentation no matter the temperature applied. At LT, when the sourdough is used the genotype effect is deficient whereas at HT there is a slight difference.

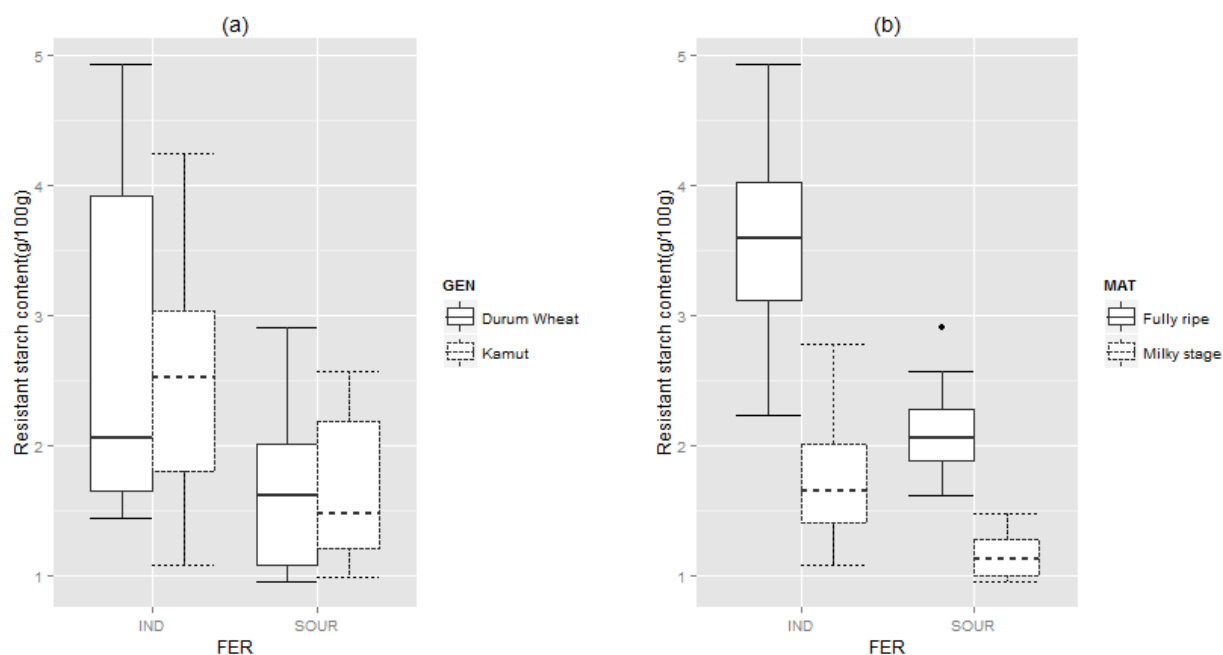


Fig.3. (a). Amount of resistant starch on genotype flour(GEN) Kamut and Durum wheat based on fermentation(FER). (b) fermentation(FER) versus the maturation stage (MAT).

Contrary to others data, fructans content in kamut was higher than durum wheat. We observed that the median of sourdough was lower than the industrial fermentation (Fig. 5.a). The durum wheat industrial data was widely ranged and a little right-skew. In the interaction FER-MAT (Fig. 5.b), median of industrial fermentation was higher than the sourdough, while the milky stage was higher compared to fully ripe only on sourdough. The median of the two maturations was the same at industrial fermentation probably due to the large distribution and the skewness of both data set. The kamut baked bread had the greater value in the industrial fermentation independently of the temperature and maturation. At the LT, the kamut milky sourdough is greater than the fully ripe, but at HT this trend is reverse (Fig. 6).

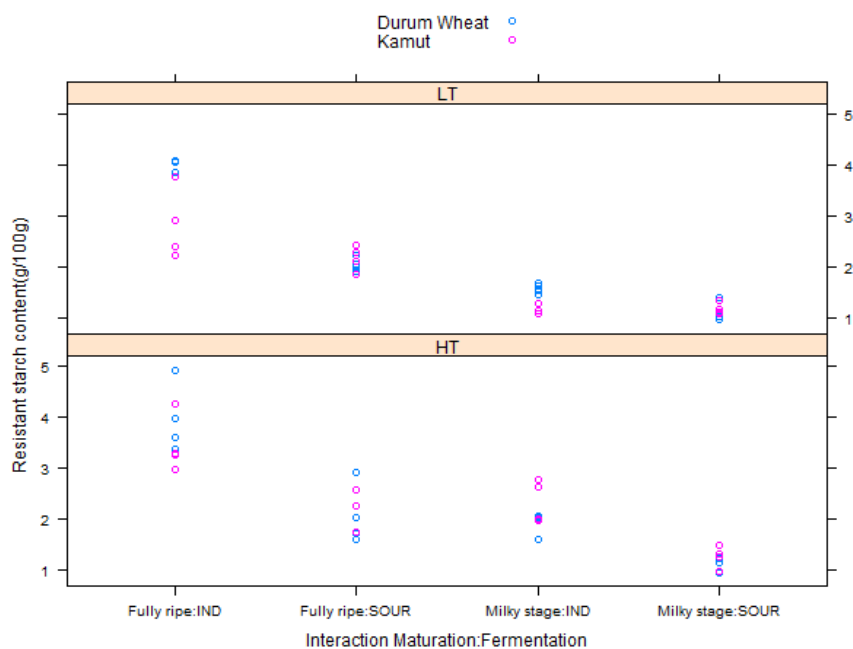


Fig.4. Amount of resistant starch on genotype flour(GEN) Kamut and Durum wheat based upon the temperature with interaction maturation : fermentation.

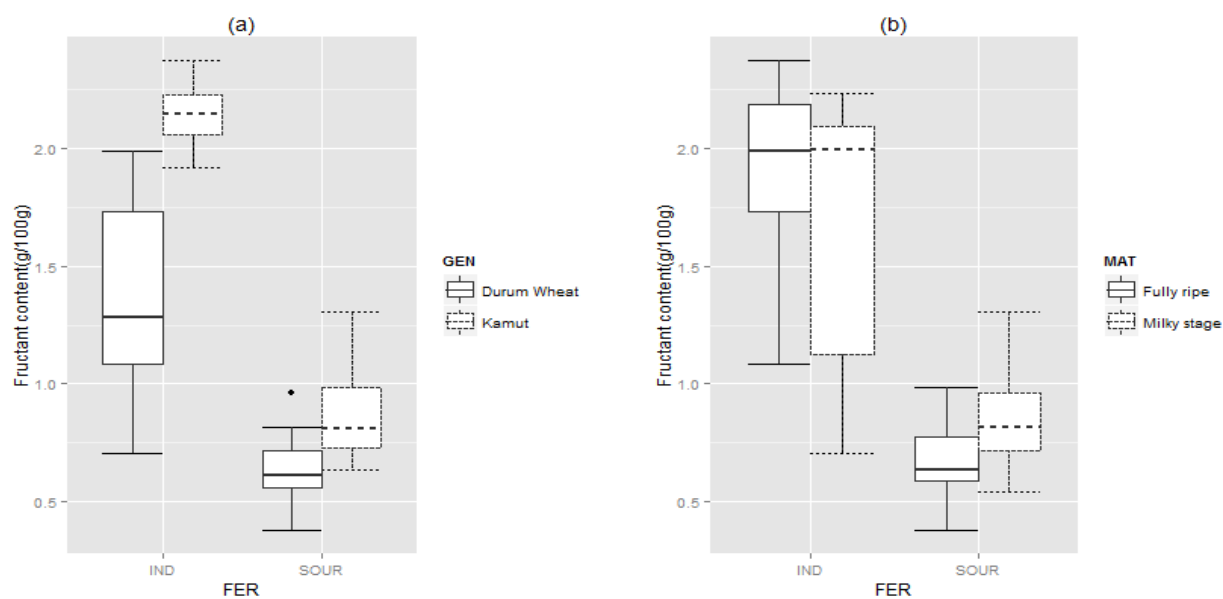


Fig.5. (a) Amount of fructans on genotype flour(GEN) Kamut and Durum wheat based on the fermentation(FER). (a) fermentation(FER) versus the maturation stage(b).

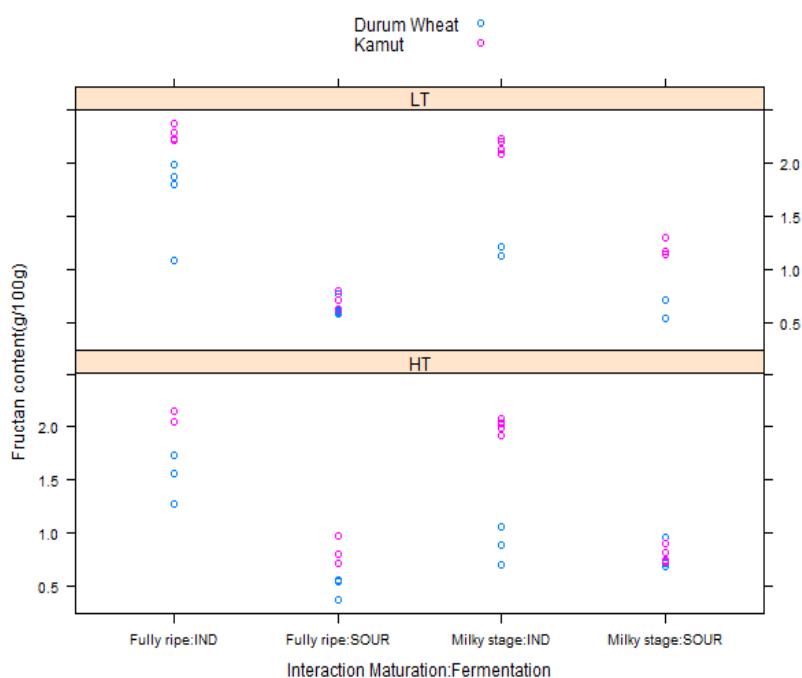


Fig.6. Amount of fructans baked breads based upon the temperature with interaction maturation : fermentation.

4. Discussion

Our results evidenced that genotype, fermentation and the kernel stage maturation had a great impact on the amount of resistant starch, fructans and β -glucans as well as their interactions with each others resulted highly significant. The baking effect was minor. We observed that sourdough process decrease the amount of β -glucans as reported also by Johansson et al.(2007): this decrease can probably due to modification of the solubility of β -glucans which became more available for degradation by endogenous enzymes. Marklinder & Johansson (1995) hypothesized that the degradation of β -glucans by endogenous β -glucanases, before the pH of sourdough drop during fermentation, can be related to the duration of the lag phase of lactic acid bacteria. As long as the lag phase of the lactic acid bacteria is as more β -glucans are degraded. Another explanation could be that the starter cultures had different abilities to degrade β -glucans. In fact, it has also been demonstrated that 22% of the lactobacilli found in the digestive tract of piglets were able to degrade β -glucans (Jonsson and Hemmingsson, 1991).

Kavita et al. (1998) reported that the resistant starch content decrease in the fermented products, which is in agreement with our results. Our findings related to resistant starch were also reported by Liljeberg et al., (1996) demonstrating that the application of a high temperature allows a better release of resistant starch. They hypothesized that RS in long-time baked products required

solubilization in alkali to make them available to amylases, indicating the presence of retrograded starch. It seems that heat is related to a decrease in the hydrolysis limit of pancreatic α -amylase and increased production of RS (Franco et al., 1995).

The amount of fructans was high in kamut (2.1g/100g) which is higher than rye bread known as the richest source (1.94 g/100 g) (Whelan et al.,(2011). Andersson et al.,(2009) showed that presence of sourdough and yeast in most of the breads can result in significant degradation of rye fructans. Similar findings for fructan content have been reported by Karppinen et al., (2003) for Finnish rye crisp breads and soft breads. They attributed the differences in fructans content in different breads to the function of fermentation, the amount of flour used in the baking and the flour itself. Several previous findings also supported the decrease in fructans content during various steps in the bread-making process. For example, Hansen et al. (2002) reported a 45% decrease in fructan content during imitated sourdough mixing, proofing and baking.

The amount of extractable fructose containing compounds in the seed decreased during seed growth (Thomas L. et al.,1987). De Gara et al.(2003) observed that, during wheat kernel maturation, protein and starch content increase whereas the mono- and di-saccharides content change it was 0.92 ± 0.02 at 13 days after anthesis (milky phase) and (0.58 ± 0.02) at 28 days after anthesis but at the complete maturation (45 days after anthesis) the value returns the same as the early milky phase. The inulin degradation during heating have been investigated by Christian and Manley-Harris(2000). Based on fructose quantification after acid hydrolysis, a decrease of the inulin content between 33 and 43% was found in inulin-containing breads after dough preparation, fermentation and baking. In general, heating of inulin under water-free conditions at temperatures higher than 135 °C induced a degradation of long-chain saccharides (A. BÖhm, 2005).

5. Conclusion

In general, breads industrially fermented retained a better quali/quantitative profile of the bioactive molecules considered in this study. The combination fully ripe flour/industrial fermentation seemed to provide a generally higher content of fructans and RS in kamut than in wheat but not of BG. When a sourdough process is adopted a more complex scenario appeared. In fact the maturation stage (milky and ripe stage) differently influences the two flours considered due to the different thermal characteristics of bioactives that influenced their content in breads. For example in fully ripe flours, the high time/temperature process of sourdough fermented bread affected fructan content lesser in Kamut than wheat bread. This brings to consider that bioactive molecules analysed here change their chemical-physical properties (including thermal sensitivity) according to their

maturation degree. These differences are not always detectable by the official analytical methods. However the different behaviour of bioactives along the process is probably to ascribe to any one of those differences. These findings may be very useful for food industry to design the right formulation and set up the best process suitable to maximise the bioactive concentration expected in foods.

References

- A. BÖhm, I. Kaiser, A. Trebstein, T. Henle.(2005). Heat-induced degradation of inulin. *Eur Food Res Technol*, 220:466–471
- A. Rakha, P. Åman, R. Andersson.(2010). Characterisation of dietary fibre components in rye products. *Food Chemistry* 119, 859–867.
- Abd-Elmoneim-O-Elkhalifa, Schiffler B, Bernhard R.(2004). Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour. *Nahrung/Food* 48(2):91–4.
- Andersson, R., Fransson, G., Tietjen, M., & Åman, P. (2009). Content and molecularweight distribution of dietary fibre components in whole-grain rye flour and bread. *Journal of Agricultural and Food Chemistry*, 57, 2004–2008.
- Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, Delzenne NM. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am J Clin Nutr* 90: 1236–1243.
- Christian TJ, Manley-Harris M.(2000). Kinetics of formation of Di-D-fructose dianhydrides during thermal treatment of inulin. *J Agric Food Chem* 48:1823–1837
- Courtin, C. and Delcour, J.A.(2002). Arabinoxylans and endoxylanases in wheat flour breadmaking, *J. Cereal Sci.*, 35, 225.
- Cummings JH, Englyst HN. (1991). Measurement of starch fermentation in the human large intestine. *Can J Physiol Pharmacol* 69:121–9.
- E. Dornez et al.(2008). Effects of genotype, harvest year and genotype-by-harvest year interactions on arabinoxylan, endoxylanase activity and endoxylanase inhibitor levels in wheat kernels. *Journal of Cereal Science* 47, 180–189.
- Franco CML, Ciacco CF, Tavares DQ. (1995). Effect of heat-moist treatment on enzymatic susceptibility. *Starke/Starch* 47(6):223–8.
- Hansen, H. B., Andreasen, M. F., Nielsen, M. M., Larsen, L. M., Bach Knudsen, K. B., Meyer, A. S.,

- et al. (2002). Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. *European Food Research and Technology*, 214, 33–42.
- Holtekjolen, A.K., Olsen, H.H.R., Faergestad, E.M., Uhlen, A.K., Knutsen, S.H., 2008. Variations in water absorption capacity and baking performance of barley varieties with different polysaccharide content and composition. *Lwt-Food Science and Technology* 41, 2085e2091.
- I. Marklinder and L. Johansson.(1995). Sourdough fermentation of barley flours with varied content of mixed-linked (1→3)(1→4)-D-glucans. *Food Microbiology*, 12, 363-371
- Jamieson, P.D., Semenov, M.A., Brooking, I.R., Francis, G.S., 1998. Sirius: a mechanistic model of wheat response to environmental variation. *European Journal of Agronomy* 8, 161–179.
- Jonsson, E. and Hemmingsson, S. (1991). Establishment in the piglet gut of lactobacilli capable of degrading mixed-linked beta-glucans. *J. Appl. Bacteriol.* 70, 512-516.
- Karppinen, S., Myllymäki, O., Forssell, P., & Poutanen, K. (2003). Fructan content of rye and rye products. *Cereal Chemistry*, 80, 168–171
- Kavita V, Varghese S, Chitra GR, Jamuna P. (1998). Effects of processing, storage time and temperature on the resistant starch of foods. *J Food Sci Technol* 35(4):299–304.
- Kevin Whelan, Olivia Abrahmsohn, Gondi J. P. David, Heidi Staudacher, Peter Irving, Miranda C. E. Lomer, Peter R. Ellis.(2011). Fructan content of commonly consumed wheat, rye and gluten-free breads. *International Journal of Food Sciences and Nutrition*, 62(5): 498–503
- Landau, S., Mitchell, R.A.C., Barnett, V., Colls, J.J., Craigon, J., Payne, R.W., 2000. A parsimonious, multiple-regression model of wheat yield response to environment. *Agricultural and Forest Meteorology* 101, 151–166
- Laura De Gara, Maria C. de Pinto, Vita M. C. Moliterni and Maria G. D'Egidio.(2003). Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *Journal of Experimental Botany*, Vol. 54, No. 381, pp. 249-258
- Liisa Johansson, Paivi Tuomainen, Heli Anttila, Hannu Rita, Liisa Virkki.(2007). Effect of processing on the extractability of oat β -glucan. *Food Chemistry* 105, 1439–1445.
- Liljeberg H, Akerberg A, Bjorck I. (1996). Resistant starch formation in bread as influenced by choice of ingredients or baking conditions. *Food Chem* 56(4):389–94.
- McCleary BV, Murphy A, Mugford DC. (2000). Measurement of total fructan in foods by enzymatic/spectrophotometric method: Collaborative study. *J AOAC Int* 83:356–364.
- McCleary, B. V. & Mugford, D. C. (1992). Interlaboratory evaluation of b-glucan analysis methods. In *The changing role of oats in human and animal nutrition. Proceedings of the Fourth International Oat Conference*, 19–23 October, Adelaide, Australia.

- McCleary, B. V., & Codd, R. (1991). Measurement of (1-3)(1-4)- β -Dglucan in barley and oats: a streamlined enzymic procedure. *Journal of the Science of Food and Agriculture*, 55, 303–312.
- McCleary, B. V., & Monaghan, D. A. (2002). Measurement of resistant starch. *Journal of the Association of Official Analytical Chemists*, 85(3), 665–675.
- Meyer D, Stasse-Wolthuis M. (2009). The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *Eur J Clin Nutr* 63:1277–1289.
- Nilsson, U., and Björck, I. (1988). Availability of cereal fructans and inulin in the rat intestinal tract. *J. Nutr.* 118:1482–1486.
- Nilsson, U., Öste, R., Jägerstad, M., and Birkhed, D. (1988). Cereal fructans: In vitro and in vivo studies on availability in rats and humans. *J. Nutr.* 118:1325-1330.
- Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, Wolvers D, Watzl B, Szajewska H, Stahl B, et al. (2010). Prebiotic effects: Metabolic and health benefits. *Br J Nutr* 104(Suppl 2):S1–S63.
- Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Açil Y, Gluer CC, Schrezenmeir J. 2007. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *J Nutr* 137(Suppl 2):838S–846S.
- Skendi, A., Biliaderis, C.G., Papageorgiou, M., Izydorczyk, M.S., 2010. Effects of two barley beta-glucan isolates on wheat flour dough and bread properties. *Food Chemistry* 119, 1159-1167.
- Slavin, J. (2004). Whole grains and human health. *Nutrition Research Reviews*, 17, 99–110.
- Sully Philippe, Luc Saulnier , Fabienne Guillon.(2006).Arabinoxylan and (1 \rightarrow 3),(1 \rightarrow 4)- β -glucan deposition in cell walls during wheat endosperm development. *Planta* 224: 449–461
- Thomas L. Housley and Craig S. T. Daughtry.(1987). Fructan Content and Fructosyltransferase Activity during Wheat Seed Growth. *Plant Physiol.* 83, 4-7
- Topping D. (2007). Cereal complex carbohydrates and their contribution to human health. *J Cereal Sci* 46, 220–229
- Topping, D. L., Anthony, M. F., & Bird, F. (2003). Resistant starch as prebiotic and synbiotic: State of the art. *Proceedings of the Nutrition Society*, 62, 171–176.

PART II. EFFECT OF WHOLE GRAIN COMPOUNDS ON THE HUMAN METABOLOME

CHAPTER I. INTRODUCTION

1. Introduction

Microbes are important for food science and technology; they are used in fermentation to improve both preservation and organoleptic properties. Nowadays, fermentation is applied for specific health attributes, for instance through the enrichment with specific vitamins or the introduction of probiotics, ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (Johan ET van Hylckama Vlieg et al., 2011). The influence of microorganisms on our ability to produce energy and nutrients from food is extended to the gastro-intestinal (GI) tract. Microbial degradation of complex carbohydrates and proteins is a key tool for food digestion but we have started to appreciate the role of the GI microbiota in human health and disease recently. This is mainly driven by recent technological advances in sequencing and other ‘omics’ technologies that offer instruments to study the complex GI microbial communities and also host responses associated to specific diet and gut microbiota perturbations (Figure 4).

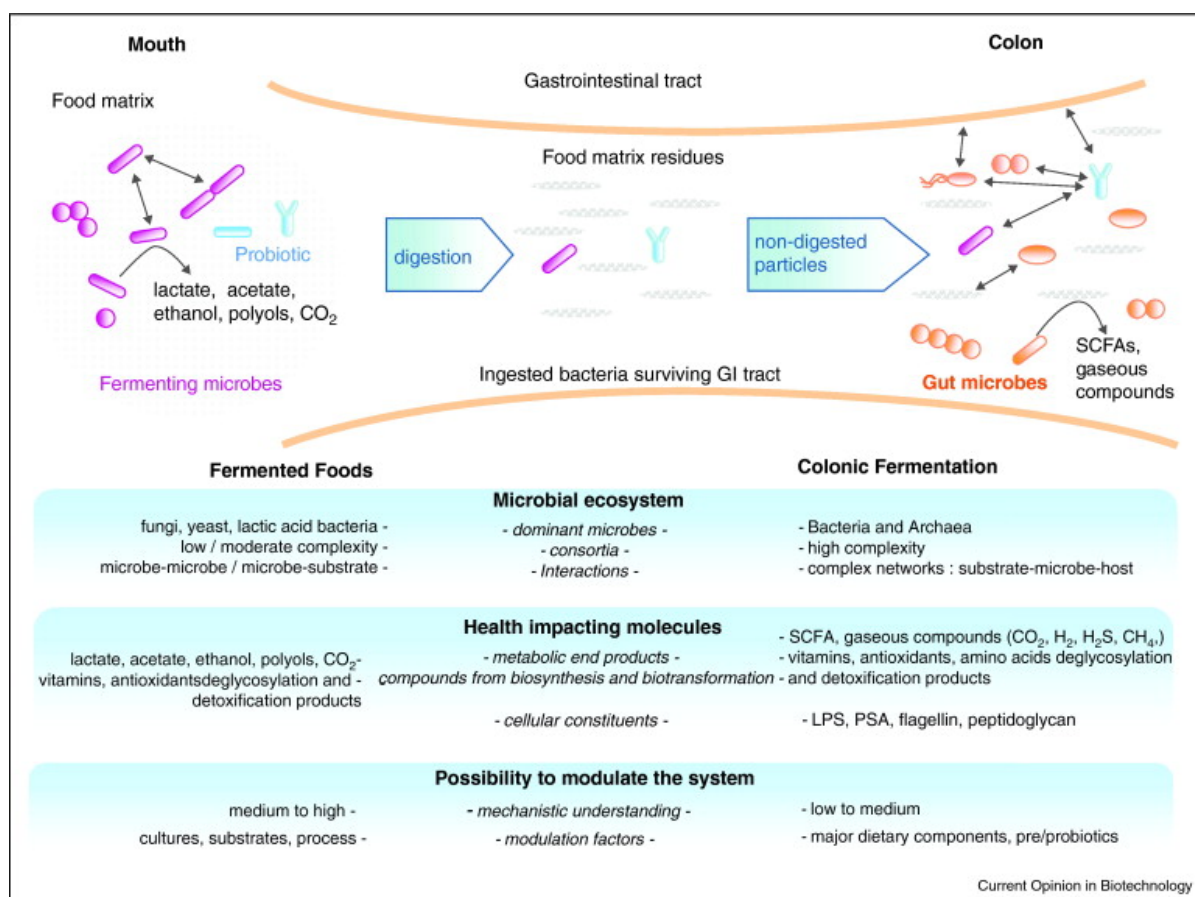


Figure 4. Fermentation in food processing and the GI tract.

2. Dietary importance and health benefits of fermented foods

The use of fermentation is extended to a broad range of food substrates and food products produced traditionally or at industrial scale (Sieuwert et al., 2008; Nout MJR.; 2009). Nowadays, fermentation technology has progressed from traditional practices and empirical science to industrialize and life science driven technology. The microbial action has an influence on the composition and nutritional status which can be valorised by the introduction of specific health beneficial attributes. Metabolism of primary carbon in fermenting microorganism is typically applied for transforming sugars into simple acids, alcohols and carbon dioxide as major end products. Besides a broad variety of secondary metabolites, including vitamins, polyols, or antioxidants, can be produced leading to specific health benefits. For example lactic acid bacteria multi-vitamin producing strains have been reported to produce different ratios of folate and cobalamin depending on the food substrate used (Santos et al., 2008; Sybesma et al., 2004). Functional attributes may also be introduced or altered through biotransformation reactions. Many vegetal products contain polyphenols or flavonoids which have health-beneficial activities. In plants these are often glycosylated which can reduce their bioavailability. Recently it has been reported that α -rhamnosidase (Avila et al., 2009; Beekwilder et al., 2009) or β -galactosidase (Pham&Shah N., 2009) activity produced by food-fermenting LAB can efficiently eliminate glycoside- residues. Finally, some fermented foods can be good sources of probiotics. Numerous well conducted, prospective, randomized, controlled, clinical trials (RCTs) are published to validate health benefits (Gareau et al., 2010). Mechanistic studies focused originally on the direct cross-talk of probiotic strains and host, specially the immune system. Analyses on the gut microbiota alterations in RCTs reported that incorporation of probiotic in diet might induce subtle modifications in the GI microbiota community structure that may play a role in the observed effect (Salonen et al., 2009).

3. Gut microbial ecology

The human gut is colonized by a several number of bacterial species (more than 800) that attain the highest concentrations in the colon (up to 10^{12} cells per gram of faeces). The colonization process in gut begins instantaneously after birth and the development and establishment of the infant's microbiota highly depend on environmental factors. The newborn infant microbiota firstly had low diversity and instability, but develops into a more constant adult-type microbiota during the first 24 months of existence (Zoetendal et al., 1998). Usually, *Bifidobacterium* species are predominant in the first months of life, in particular in breast-fed infants (up to 90% of the total faecal bacteria) as a result of the bifidogenic effect of breast milk, whereas a more-diverse microbiota is found in formula-fed infants, weaning children and adults (Gueimonde et al., 2006). Metagenomic analyses

reported that in adults and weaning children the main components of the colonic microbiota are *Bacteroides*, followed by numerous genera belonging to the division *Firmicutes*, such as *Eubacterium*, *Ruminococcus* and *Clostridium*, and the genus *Bifidobacterium*. However, in babies the genus *Bifidobacterium* is dominant as well as a few genera from the family *Enterobacteriaceae*, such as *Escherichia*, *Raoultella*, and *Klebsiella* (Kurokawa et al., 2007). The composition of this bacterial ecosystem is dynamic and susceptible depends on dietary factors and various disease conditions (Xu et al., 2007; Sanz et al., 2008).

Disorders occurring in this ecosystem by environmental factors such as diet, pathogens or antibiotic treatment coupled with genetic pre-depositions in the host, may induce to 'dysbiosis' and impaired activity that can have a negative impact on health (Hawrelak & Myers., 2004). From all undigested dietary components, particularly those which are non-digestible by host enzymes, such as dietary fibres, or those escaping from upper digestion and absorption, such as overeaten proteins, can be metabolized and transformed by the gut microbiota (Zhang et al., 2010). This last authors also reported that diet have important role in shaping gut microbiota, prevailing host genetics. About 60 % of total gut microbiota variations might be due to change of diet whereas only about 10% can be attributed to a host genetic variation. Main changes in diet directly influence the microbiota composition and specific phylogenetic groups may appear within a few days in response to a change in carbohydrate and protein intake (Walker et al., 2010). Studies on metagenomic sequencing of the gut microbiome (collective genome of gut microbiota) observed a huge genetic range with vast arrays of genes for breakdown and transformation of diverse dietary components (Nelson et al., 2010; Qin et al., 2010; Gill et al., 2006). Polysaccharides are mainly degraded by Gram-negative anaerobes belonging to the genus *Bacteroides* (Salysers & Leedle 1983). Starch fermentation yields high levels of butyrate, while with a more oxidized substrate such as pectin; more acetate is produced (Englyst et al. 1987; Scheppach et al. 1988).

4. Roles of the gut microbiota in host physiology and health

The gut microbiota has various functions involving protective, immune and metabolic, which altogether have vast influence on the nutritional and health status of the host. The native gut microbiota and transient bacteria (food-associated and probiotics) are recognized to have an influence on the development and regulation of the host's defences, of immune and non-immune nature, through interaction with the epithelium and the gut-associated lymphoid tissue (Sanz et al., 2009). The intestinal epithelium represents a physical hurdle that controls the transcellular and paracellular transit of exogenous substances and damages the access of most of luminal antigens;

this hurdle is reinforced by the mucus layer included by glycoproteins (mucins) and the synthesis of antimicrobial peptides and other secretions (bile, acids, enzymes, etc.). The commensal microbiota are element of this first defence action, and contributes to modulation of paracellular permeability, mucin gene expression by goblet cells and secretion of antimicrobial peptides (defensins and angiogenins) by Paneth cells of intestine. Furthermore, the gut microbiota is indispensable to the postnatal development of the immune system, influencing the content of lamina propria T cells, immunoglobulin A producing B cells, intraepithelial T cells and serum immunoglobulin levels (Taskalová-Hogenová et al., 2004). According to these protective and immunomodulatory functions, some probiotic strains are known for their beneficial effects on the cure of acute diarrhoea, prevention of antibiotic associated-diarrhoea, eradication of *Helicobacter pylori* infection together with antibiotics and in prevention of atopic eczema in humans (Sanz et al., 2009; Kalliomäki et al., 2007) .

The gut microbiota also influences the host metabolism, supplying additional enzymes and regulating the expression of genes implicated in the utilization of carbohydrates and lipids, and in drugs bioconversion (Hooper et al., 2002; Gill et al., 2006; Wilson & Nicholson., 2009). The number of genes of the collective genome (microbiome) of the microbiota goes beyond those of the human genome, encoding extra metabolic attributes (Gill et al., 2006; Turnbaugh et al., 2007). Genomic and physiologic studies reported that gut microbiota has enzymes specialized in the utilization of undigested carbohydrates and host-derived glycoconjugates (e.g. mucin), deconjugation and dehydroxylation of bile acids, cholesterol reduction, biosynthesis of vitamins (K and B group) and isoprenoids and metabolism of amino acids and xenobiotics (Kurokawa et al., 2007; Hooper et al., 2002; Gill et al., 2006).

In the microbiome is predominantly found genes implicated in carbohydrate metabolism and uptake, because complex polysaccharides are the main energy source for the colonic microbiota (Kurokawa et al., 2007). The genome sequence of *Bifidobacterium longum* also provides various number of predicted proteins (more than 8%) associated to the catabolism of oligo- and polysaccharides liberated from indigestible plant polymers (Schell et al., 2002) . several of the most dominant bacterial enzymes implicated in the degradation of complex polysaccharides and xenobiotics are β -glycosidases and β -glucuronidases, which could have both beneficial and harmful roles (Dabek et al., 2008). Glycosidase activities occurring in the human colonic microbiota operates on plant glucosides assisting to nutrient utilization and, in some cases, to the production of biologically active aglycones with other health benefits (e.g. from isoflavones). The utilization of complex dietary polysaccharides by the microbiota seems to contribute to harvest energy from the

diet, which may represent 10% of the daily energy supply (Flint et al., 2008). Fermentation of non-digested polysaccharides reaching the colon release short chain fatty acids (SCFAs) as end products, principally acetate, propionate and butyrate and gases such as carbon dioxide, hydrogen and methane (Hijova & Chmelarova., 2007; Samuel et al., 2008). Resulting from an intense metabolic activity of the gut microbiota on mean 13 L of hydrogen is generated during colonic fermentation on a daily basis (Nakamura et al., 2010). The common is reused by bacterial during cross-feeding reactions, amongst others leading to the production of methane and hydrogen sulphide. Part of the gases is enclosed in the intestine and needs to be excreted. Dysfunctions in the physiological processes leading to the management of these fermentative gases may possibly provoke generation of abdominal distension and bloating (Serra et al., 2010). Short-chain fatty acids (SCFAs) are an essential group of metabolites. While acetate and propionate are used by the portal vein and metabolized by the liver as an energy source, butyrate is quickly wrapped up by the host as the major energy source for colonocytes (Thibault et al., 2010). In the liver acetate seems to contribute to lipid and cholesterol synthesis whereas propionate can inhibit the effects of acetate van Hylckama Vlieg et al., 2011)

Rarely, β -glycosidases and β -glucuronidases generally release toxins and mutagens that have been glucuronated in the liver and secreted into the gut with the bile. This resulting to the accumulation of high local concentrations of carcinogenic compounds within the gut, so increasing the risk of carcinogenesis. Moreover, reuptake of the deconjugated compound from the gut and re-glucuronidation in the liver driven to an enterohepatic circulation of xenobiotic compounds, which increments their retention time in the body. In the colonic microbiota, bacterial β -glucosidases appears to be more common than β -glucuronidases. Studies performed in 40 bacterial strains, which are typical of predominant bacteria in human faeces, reported that more than half of the low G + C% Gram-positive *Firmicutes* have β -glucosidase activity, whereas β -glucuronidase activity is only found in some *Firmicutes*, within the clostridial clusters XIVa and IV (Dabek et al., 2008). The major part of *Bifidobacterium* spp. and *Bacteroides thetaiotaomicron* present β -glucosidase activity. Furthermore, the intensity of exposure to glycosides in the colon, which is related on the nature of diet consumed, possibly will influence the induction of enzyme activity levels in some components of the gut microbiota and, thus, impacting their functions (Dabek et al., 2008). Specific glycosidases (xylanases, arabinofuranosidases and xylosidases) needed for complete degradation of complex polysaccharides existing in plant cell walls, such as arabinans and arabinoxylans, are also encoded by the total faecal microbiota and by strains of the principal bacterial genera (*Bifidobacterium* and *Bacteroides*) (Gueimonde et al., 2007; Grootaert et al., 2009). *Bifidobacterium longum* subsp.

infantis ATCC 15697, isolated from the newborn gut, is also contained genes and enzymes permitting the favoured consumption of small portion of oligosaccharides, which correspond to 63.9% of the total human milk oligosaccharides accessible (LoCascio et al., 2007; Sela et al., 2008). Additionally, genes coding for an endo- α -N-acetylgalactosaminidase and a 1,2- α -L-fucosidase, which hydrolyse high-molecular weight mucin, are found in some *Bifidobacterium bifidum* strains (Ruas-Madiedo et al., 2008).

In the metabolism of cholesterol and bile acids, Gut bacterial enzymes are also implicated. Cholesterol is probably converted to coprostanol by the commensal microbiota, raising its discharge in faeces. In the liver, Bile acids are produced from cholesterol mainly as the primary bile acids, cholic acid and chenodeoxycholic acid. Gut bacteria can also reduce these acids into different types of secondary bile acids by catalysing their deconjugation and dehydroxylation, thereby restraining the solubilisation and absorption of dietary lipids all over the intestine (Ridlon et al., 2006). Nevertheless, these activities also may contribute to the production of secondary bile acids, some of which (deoxycholic acid and lithocholic acid) appears to be possible carcinogens. *Bacteroides intestinalis* and secondarily *Bacteroides fragilis* and *Escherichia coli* are probably engaged in the production of secondary bile acids in the colon (Fukuya et al., 2009).

The supply of amino acids required by humans is also present in the metabolic activity of the microbiota. When fermentable carbohydrate substrates (e.g. non-starch polysaccharides, resistant starch and oligosaccharides) are available, colonic bacteria grow and dynamically synthesize protein, which may be a source of amino acids for the host (Cummings & Macfarlane., 1997). Even though it is not easy to measure the protein synthesis and turnover within the large intestine, at least from 1 to 20% of circulating plasma lysine and threonine in adult human is originated from gut microbiota, as estimated by using characterized amino acids (Hooper et al., 2002). However, the metabolic activities of the microbiota implicated in the degradation of food source nitrogen compounds (e.g. nitro compounds, sulphur-containing compounds and amino acids) may lead to the production of potentially carcino-genetic substances (Rowland IR., 2009).

The gut microbiota also modulates the expression of genes engaged in the processing and absorption of dietary carbohydrates and complex lipids by the host, which altogether lead to body weight gain and raised fat storage. Gut population by commensal bacteria raises the expression of an intestinal monosaccharide transporter and key enzymes (acetyl-CoA carboxylase and fatty acid synthase) of *de novo* fatty acid biosynthetic pathways (Hooper et al., 2001; Bäckhed et al., 2004). The colonization of germ-free mice also decreases the levels of circulating fasting-induced adipose factor (Fiaf) and the skeletal muscle and liver levels of phosphorylated AMP-activated protein

kinase, having a role in fat storage (Bäckhed et al., 2007). Moreover, comparisons between germ-free and colonized rat showed that the gut microbiota have an effect on levels of xenobiotic-metabolizing enzymes in large intestine and liver, comprising glutathione transferases, gastrointestinal glutathione peroxidase, epoxide hydrolases, N-acetyltransferases, and cytochrome P450 activities, which might modify the host ability to detoxify different compounds (Overvik et al., 1990; Meinel et al., 2009). Consequently, the intestinal tract is populated by a complex microbiota that promotes strategies to regulate nutrient possession and utilization in symbiosis with the host and in response to the diet. The biochemical activity of this complex ecosystem produces healthy and also potentially harmful compounds from the diet and their equilibrium is indispensable to preserve a healthy status. This might be done incorporating probiotic bacteria and other functional food components in the daily diet.

5. Prebiotics and gut microbiota

Prebiotics are indigestible food components, mostly oligosaccharides, which beneficially affect the host by stimulating growth, activity or both of specific intestinal bacteria (Roberfroid., 2007). The criteria that have to fulfill a prebiotic include, (1) resistance to gastric acidity and mammalian enzymes; (2) accessibility for fermentation by gut microbiota; and (3) ability to stimulate the growth and/or activity of beneficial intestinal bacteria. The positive effects of prebiotics include the control of intestinal transit time and bowel habits, and decrease of risk of atherosclerosis, osteoporosis, obesity, type-2 diabetes, cancer, infections and allergies, though their efficiency in humans is still uncertain (Roberfroid., 2007). The biological properties of prebiotics mostly rely on their impact on the gut microbiota composition and derived metabolites; though some roles could be a result of their own structure and direct action (e.g. inhibition of pathogen adhesion by homology with bacterial receptors).

Galacto-oligosaccharides (GOS) and inulin-derivatives (e.g. fructo-oligosaccharides [FOS]) are the mostly prebiotics known in Europe. GOS are indigestible oligosaccharides obtained from lactose that are found naturally in human milk and consist of chains of galactose monomers. These prebiotics offer beneficial effects in the gastrointestinal tract by stimulating growth of specific components of the intestinal microbiota (e.g. bifidobacteria). GOS alone or combined with FOS are mostly incorporated to infant formula to develop the dominance of a microbiota composition comparable to that resulting from breast-feeding during both milk feeding and the weaning period. Consumption of food enriched with prebiotics leads to an increase of the total amount of faecal bifidobacteria and favour a *Bifidobacterium* species composition close to that of breast-fed

infants. The effects of these prebiotics on immune functions can be derived from the induced changes on the gut microbiota and/or to the effects of the generated SCFAs through binding to SCFA receptors on leucocytes (Watzl et al., 2005). Feeding Studies using long-chain inulin demonstrated beneficial effects on bowel inflammation dropping the production of pro-inflammatory biomarkers, simultaneously with an increase of gut bifidobacteria and lactobacilli (Videla et al., 2001; Welters et al., 2002). SCFA regulates also intestinal fat absorption since butyrate, for instance, impairs lipid transport *in vitro* in Caco-2 cells (Marcil et al., 2003).

6. Metabolic products of the gut fermentation process

SCFAs produced by the gut microbiota are probably around ten percent of the energy extracted from food in human. In fact, the aptitude of the gut microbiota to convert otherwise indigestible food components into molecules that serve as an energy source for the host was proposed to be involved in obesity pathogenesis due to an excess energy intake (Bäckhed et al., 2004; Bäckhed et al., 2007). Besides his role in energy metabolism butyrate also plays a role in maintaining the integrity of gut mucosa. Butyrate can decrease risk for colon cancer development by reducing cell proliferation and stimulating cell differentiation and it is also related with a maturation of enteric neurons (Soret et al., 2010; Hamer et al., 2008). Furthermore, SCFAs are important modulators of the immune system (Maslowski et al., 2009), for example they might assist resolve chronic inflammation associated with IBD by interacting with G-protein-coupled receptors such as GPR43, lately renamed as Free Fatty Acid Receptor 2 (FFAR2) (Karaki et al., 2008). As organic acids, SCFAs play a role in the control of the luminal pH and as a result influence the levels of bacteria sensitive to low pH including some pathogens.

Besides SCFAs, other fermentation products might also promote the host health. Recently there had been wide range of health modulating metabolites described including anti-inflammatory factors produced by commensals such as *Faecalibacterium prausnitzii* (Sokol et al., 2008) and compounds able to modulate intestinal pain (Rousseaux et al., 2007). This also comprises essential nutrients such as vitamin K2 important for the modulation of bone mineralisation and blood coagulation (Conly et al., 1994; Van et al., 2009), and vitamin B12 involved in nervous system development (Santos et al., 2008; Dror & Allen 2008). The gut microbiota also produces γ -aminobutyric acid (GABA), a neurotransmitter implicated in the regulation of the central nervous system, relief of hypotension and diuresis (Huang et al., 2007). Other active molecules produced are, the polysaccharide A (PSA) which is reported to decrease the production of proinflammatory cytokine levels, neutrophil infiltration, and epithelial cell hyperplasia (Mshvildadze et al., 2008; Forsythe et al., 2010) and

conjugated linoleic acid (CLA) that modulates the immune system (Devillard et al., 2007; Ross et al., 2010). Administration of a Bifidobacterium strain able to produce CLA has been shown to induce a change of the host fatty acid composition (Wall et al., 2009) indicating the possibility to use the gut microbiota as a lever to modulate lipid metabolism. Other acidic products of fermentation are present in the large gut, such as the branched-chain fatty acids, isobutyrate, 2-methylbutyrate and isovalerate, which are products of amino acid fermentation, whilst other organic acids, including the electron sink products lactate and succinate, accumulate to a lesser degree (Cummings et al. 1987). Further acidic, neutral or basic products of the hind gut fermentation include phenols, indoles, amines and ammonia (Drasar & Hill., 1974), which are generated during the catabolism of amino acids

7. Phytochemicals and gut microbiota

Phytochemicals are bioactive non-nutrient plant compounds found in fruits, vegetables, grains, and other plant foods, whose consumption has been correlated to reduction in risk of major chronic diseases (Liu, 2004). They can be divided according to common structural features into carotenoids, phenolics, alkaloids and nitrogen-containing and organosulphur compounds. Phenolics, flavonoids and phytoestrogens are particularly important due of their potential effects as antioxidant (Hertog et al., 1993), antiestrogenic (Yuan et al., 2007), anti-inflammatory and immunomodulatory (Park et al., 2007; Ruiz & Haller., 2006), cardioprotective and anticarcinogenic (Liu., 2004; Hertog et al., 1993) compounds. The bioaccessibility and effects of polyphenols are mostly related to their transformation by specific constituents of the gut microbiota through esterase, glucosidase, demethylation, dehydroxylation and decarboxylation activities (Aura, 2008). Numerous dietary polyphenols are glycosides resulted from transformation into aglycones by gut bacterial glycohydrolases, thereby changing their bioavailability and influencing positively or negatively their activities and functional effects on the mammalian tissues (Gee & Johnson., 2001; Scalbert et al., 2002). Polyphenols are usually found in plant foods as a bound form, most frequently conjugated as glycosides, which are metabolized by gut microbiota leading to production of aglycones (Bowey et al., 2003; Larrosa et al., 2009). The microbiota metabolites of polyphenols are well absorbed in the intestine, and their enterohepatic circulation guarantees that the dwelling time in plasma for the metabolites is extended compared to that of their parent compounds, and finally are excreted via urine. The gut microbiota mainly contributes to the production of active isoflavone metabolites with oestrogen-like activity; in addition, the produced metabolites have different anti-inflammatory properties (Park et al., 2007). likewise, the flavonoid quercetin produced by gut

microbial enzymes exercises a higher effect in the down-regulation of the inflammatory responses than the glycosylated form present in vegetables (quercitrin or 3-rhamnosylquercetin) (Comalada et al., 2005). This effect is exerted by inhibiting cytokine and inducible nitric oxide synthase expression via inhibition of the NF-kappaB pathway both *in vitro* and *in vivo* (Comalada et al., 2005). On the contrary, the ellagitannin punicalagin which is the most potent antioxidant present in pomegranate juice is widely metabolized to hydroxy-6H-dibenzopyran-6-one derivatives, which did not have significant antioxidant activity compared to punicalagin (Cerdeira et al., 2004).

Phytochemicals and their derived products can also have an effect on the intestinal ecology as a significant amount of them are not fully absorbed and are metabolised in the liver, excreted throughout the bile as glucuronides and accumulated in the ileal and colorectal lumen (Bazzocco et al., 2008). For instance, the consumption of flavonol-rich foods has been demonstrated to change the composition of the gut microbiota, exerting prebiotic-like effects (Tzonis et al., 2008). Dietary phenolics and their metabolites that are not absorbed exert antimicrobial or bacteriostatic activities (Lee et al., 2006). These metabolites selectively inhibit pathogen growth and stimulate the growth of commensal bacteria, including also some known probiotics (Lee et al., 2006; Larrosa et al., 2009), hence influencing the microbiota composition. Plant phenolic compounds from olives (Medina et al., 2009), tea (Lee et al., 2006), wine (Larrosa et al., 2009) and berries (Puupponen-Pimia et al., 2005; Nohynek et al., 2006) have shown antimicrobial properties. Tea phenolics have reported to inhibit the increase of *Bacteroides* spp., *Clostridium* spp. (*C. perfringens* and *C. difficile*), *E. coli* and *Salmonella typhimurium* (Lee et al. 2006). The intensity of inhibition was associated to the chemical structure of the compound and bacterial species. In general, caffeic acid has a more significant inhibitory effect on pathogen growth than epicatechin, catechin, 3-*O*-methylgallic acid, and gallic acid. Another *in vitro* research reported that (+)-catechin increased the counts of *Clostridium coccooides*–*Eubacterium rectale* group and *E. coli*, but inhibited those of *Clostridium histolyticum* (Tzonis et al., 2008). The effects of (–)-epicatechin were less obvious increasing the growth of *Clostridium coccooides*–*Eubacterium rectale* group (Tzonis et al., 2008). Interestingly, the growth of beneficial bacteria (*Bifidobacterium* spp. and *Lactobacillus* spp.) was relatively unaffected or favoured (Tzonis et al., 2008; Lee et al. 2006). Phenolics and flavonoids can also decrease the adhesion ability of *L. rhamnosus* to intestinal epithelial cells (Parkar et al., 2008). Tea catechins have also been demonstrated to change mucin content of the ileum which might regulate bacterial adhesion and colonization (Ito et al., 2008). Thus, polyphenols seem to have potential health benefits in the modulation of gut microecology.

8. REFERENCES

- Aura AM.(2008). Microbial metabolism of dietary phenolic compounds in the colon. *Phytochem Rev* 7:407–29.
- Avila M, Jaquet M, Moine D, Requena T, Pelaez C, Arigoni F, Jankovic I.(2009). Physiological and biochemical characterization of the two {alpha}-L-rhamnosidases of *Lactobacillus plantarum* NCC245. *Microbiology* 155:2739-2749.
- Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al.(2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 101:15718–23.
- Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. (2007).Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 104:979–84.
- Bazzocco S, Mattila I, Guyot S, Renard CM, Aura AM.(2008). Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. *Eur J Nutr* 47:442–52.
- Beekwilder J, Marcozzi D, Vecchi S, de Vos R, Janssen P, Francke C, van Hylckama Vlieg J, Hall RD.(2009). Characterization of rhamnosidases from *Lactobacillus plantarum* and *Lactobacillus acidophilus*. *Appl Environ Microbiol* 75:3447-3454.
- Bowey E, Adlercreutz H, Rowland I.(2003).Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human associated rats. *Food Chem Toxicol* 41:631–6.
- Cerda B, Espín JC, Parra S, Martínez P, Tomás-Barberán FA.(2004). The potente in vitro antioxidante ellagitannins from pomegranate juice are metabolised into bioavailable but poor antioxidant hydroxy-6H-dibenzopyran-6-one derivatives by the colonic microflora of healthy humans. *Eur J Nutr* 43:205–20.
- Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Gálvez J, et al.(2005). In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. *Eur J Immunol* 35:584–92.
- Conly JM, Stein K, Worobetz L, Rutledge-Harding S.(1994). The contribution of vitamin K2 (menaquinones) produced by the intestinal microflora to human nutritional requirements for vitamin K. *Am J Gastroenterol* 89:915-923.
- Cummings JH, Macfarlane GT.(1997). Role of intestinal bacteria in nutrient metabolism. *JPEN J Parenter Enteral Nutr* 21(November–December (6)):357–65.
- Dabek M, McCrae SI, Stevens VJ, Duncan SH, Louis P.(2008). Distribution of beta- glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria.

FEMS Microbiol Ecol 66:487–95.

Devillard E, McIntosh FM, Duncan SH, Wallace RJ.(2007). Metabolism of linoleic acid by human gut bacteria: different routes for biosynthesis of conjugated linoleic acid. J Bacteriol 189:2566-2570.

Drasar, B. S. & Hill, M .J. (1974). Human Intestinal Flora. London : Academic Press.

Dror DK, Allen LH.(2008). Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev 66:250-255.

Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA.(2008). Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat Rev Microbiol 6:121–31.

Forsythe P, Sudo N, Dinan T, Taylor VH, Bienenstock J.(2010). Mood and gut feelings. Brain Behav Immun 24:9-16.

Fukuya S, Arata M, Kawashima H, Yoshida D, Kaneko M, Minamida K, et al. (2009). Conversion of cholic acid and chenodeoxycholic acid into their 7-oxo derivatives by *Bacteroides intestinalis* AM-1 isolated from human feces. FEMS Microbiol Lett 293:263–70.

Gareau MG, Sherman PM, Walker WA.(2010). Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol 7:503-514.

Gee JM, Johnson IT.(2001). Polyphenolic compounds: interactions with the gut and implications for human health. Curr Med Chem 8:1245–55.

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE.(2006). Metagenomic analysis of the human distal gut microbiome. Science 312:1355-1359.

Grootaert C, Van den Abbeele P, Marzorati M, Broekaert WF, Courtin CM, Delcour JA, et al.(2009). Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. FEMS Microbiol Ecol 69:231–42

Gueimonde M, Noriega L, Margolles A, de los Reyes-Gavilán CG.(2007). Induction of alpha-L-arabinofuranosidase activity by monomeric carbohydrates in *Bifidobacterium longum* and ubiquity of encoding genes. Arch Microbiol 187:145–53.

Gueimonde M, Salminen S, Isolauri.(2006). Presence of specific antibiotic (tet) resistance genes in infant faecal microbiota. FEMS Immunol Med Microbiol;48:21–5.

Hamer H, Jonkers D, Venema K, Vanhoutvin S, Troost F, Brummer R.(2008). The role of butyrate on colonic function. Aliment Pharmacol Ther 27:104-119.

Hawrelak J, Myers S.(2004). The causes of intestinal dysbiosis: a review. Altern Med Rev 9:180-197.

- Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D.(1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* 342:1007–11.
- Hijova E, Chmelarova A.(2007). Short chain fatty acids and colonic health. *Bratisl Lek Listy* 108:354-358.
- Hooper LV, Midtvedt T, Gordon JI.(2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* 22:283–307.
- Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI.(2001). Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 291:881–4.
- Huang J, Le-He M, Wu H, Lin DQ.(2007). Biosynthesis of gammaaminobutyric acid (GABA) using immobilized whole cells of *Lactobacillus brevis*. *World J Microbiol Biotechnol* 23:865-871.
- Ito Y, Ichikawa T, Iwai T, Saegusa Y, Ikezawa T, Goso Y, et al.(2008).Effects of tea catechins on the gastrointestinal mucosa in rats. *J Agric Food Chem* 56:12122–6.
- Johan ET van Hylekama Vlieg, Patrick Veiga, Chenhong Zhang, Muriel Derrien¹ and Liping Zhao.(2011)Impact of microbial transformation of food on health—from fermented foods to fermentation in the gastro-intestinal tract. *Current Opinion in Biotechnology* 22:211–219
- Kalliomäki M, Salminen S, Poussa T, Isolauri E.(2007). Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *J Allergy Clin Immunol* 119(4):1019–21.
- Karaki S, Tazoe H, Hayashi H, Kashiwabara H, Tooyama K, Suzuki Y, Kuwahara A.(2008). Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J Mol Histol* 39:135-142.
- Kurokawa K, Itoh T, Kuwahara T, Oshima K, Toh H, Toyoda A, et al.(2007). Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res* 14:169–81.
- Larrosa M, Luceri C, Vivoli E, Pagliuca C, Lodovici M, Moneti G, et al.(2009). Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. *Mol Nutr Food Res* 53:1044–54.
- Larrosa M, Yanéz-Gascón MJ, Selma MV, González-Sarriás A, Toti S, Cerón JJ, et al.(2009). Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. *J Agric Food Chem* 57:2211–20
- Lee HC, Jenner AM, Low CS, Lee YK.(2006). Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Res Microbiol* 157:876–84.
- Liu RH.(2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 134:3479S–85S.

- LoCascio RG, Ninonuevo MR, Freeman SL, Sela DA, Grimm R, Lebrilla CB, et al. (2007). Glycoprofiling of bifidobacterial consumption of human milk oligosaccharides demonstrates strain specific, preferential consumption of small chain glycans secreted in early human lactation. *J Agric Food Chem* 55:8914–9.
- Marcil V, Delvin E, Garofalo C, Levy E.(2003). Butyrate impairs lipid transport by inhibiting microsomal triglyceride transfer protein in Caco-2 cells. *J Nutr* 133:2180–3.
- Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D et al.(2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461:1282-1286.
- Medina E, Garcia A, Romero C, de Castro A, Brenes M.(2009). Study of the anti-lactic acid bacteria compounds in table olives. *Int J Food Sci Technol* 7:1286–91.
- Meinl W, Szczesny S, Brigelius-Flohé R, Blaut M, Glatt H.(2009). Impact of gut microbiota on intestinal and hepatic levels of phase 2 xenobiotic-metabolizing enzymes in the rat. *Drug Metab Dispos* 37:1179–86.
- Mshvildadze M, Neu J, Mai V.(2008). Intestinal microbiota development in the premature neonate: establishment of a lasting commensal relationship? *Nutr Rev* 66:658-663.
- Nakamura N, Lin H, McSweeney C, Mackie R, Gaskins H.(2010). Mechanisms of microbial hydrogen disposal in the human colon and implications for health and disease. *Annu Rev Food Sci Technol* 1:363-395.
- Nelson KE, Weinstock GM, Highlander SK, Worley KC, Creasy HH, Wortman JR, Rusch DB, Mitreva M, Sodergren E, Chinwalla AT et al.(2010). A catalog of reference genomes from the human microbiome. *Science* 328:994-999.
- Nohynek LJ, Alakomi HL, Kahkonen MP, Heinonen M, Helander IM, Oksman- Caldentey KM, et al. (2006). Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. *Nutr Cancer* 54:18–32.
- Nout MJR.(2009). Rich nutrition from the poorest —cereal fermentations in Africa and Asia. *Food Microbiol*, 26:685-692.
- Overvik E, Lindeskog P, Midtvedt T, Gustafsson JA.(1990). Mutagen excretion and cytochrome P-450-dependent activity in germfree and conventional rats fed a diet containing fried meat. *Food Chem Toxicol* 28:253–61.
- Park JS, Woo MS, Kim DH, Hyun JW, Kim WK, Lee JC, et al.(2007). Anti-inflammatory mechanisms of isoflavone metabolites in lipopolysaccharide-stimulated microglial cells. *J Pharmacol Exp Ther* 320:1237–45.

- Parkar SG, Stevenson DE, Skinner MA.(2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. *Int J Food Microbiol* 124:295–8
- PhamT, Shah N.(2009). Performance of starter in yogurt supplemented with soy protein isolate and biotransformation of isoflavones during storage period. *J Food Sci* 74:M190-M195.
- Puupponen-Pimia R, Nohynek L, Hartmann-Schmidlin S, Kähkönen M, Heinonen M, Määttä-Riihinen K, et al.(2005). Berry phenolics selectively inhibit the growth of intestinal pathogens. *J Appl Microbiol* 98:991–1000
- Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T et al.(2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464:59-65.
- Ridlon JM, Kang DJ, Hylemon PB.(2006). Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* 47:241–59
- Roberfroid MB.(2007). Prebiotics: the concept revisited. *J Nutr* 137:830S–7S.
- Ross RP, Mills S, Hill C, Fitzgerald GF, Stanton C.(2010). Specific metabolite production by gut microbiota as a basis for probiotic function. *Int Dairy J* 20:269-276.
- Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M et al.(2007). *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* 13:35-37.
- Rowland IR.(2009). The role of the gastrointestinal microbiota in colorectal cancer. *Curr Pharm Des* 15:1524–7.
- Ruas-Madiedo P, Gueimonde M, Fernández-García M, de los Reyes-Gavilán CG, Margolles A.(2008). Mucin degradation by *Bifidobacterium* strains isolated from the human intestinal microbiota. *Appl Environ Microbiol* 74:1936– 40.
- Ruiz PA, Haller D. (2006). Functional diversity of flavonoids in the inhibition of the proinflammatory NF-kappaB, IRF, and Akt signaling pathways in murine intestinal epithelial cells. *J Nutr* 136:664–71
- Salonen A, Palva A, de Vos W.(2009). Microbial functionality in the human intestinal tract. *Front Biosci* 14:An extensive review of the composition and functionality (transcriptome, proteome, and metabolome) of the intestinal microbiota.
- Salyers, A. & Leedle, A.Z. (1983). Carbohydrate utilization in the human colon. In *Human Intestinal Microflora in Health and Disease* ed. Hentges, D.J. pp. 129-146. London: Academic Press.
- Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC,

- Crowley J, Yanagisawa M et al. (2008). Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor Gpr41. *Proc Natl Acad Sci USA* 105:16767-16772.
- Santos F, Wegkamp A, de Vos WM, Smid EJ, Hugenholtz J. (2008). High level folate production in fermented foods by the B12 producer *Lactobacillus reuteri* JCM1112. *Appl Environ Microbiol*, 74:3291-3294.
- Sanz Y, De Palma G. (2009). Gut microbiota and probiotics in modulation of epithelium and gut-associated lymphoid tissue function. *Int Rev Immunol* 28:397-413.
- Sanz Y, Sánchez E, De Palma G, Medina M, Marcos A, Nova E. (2008). Indigenous gut microbiota, probiotics, and coeliac disease. In: Overton Linda T, Ewente MR, editors. *Child nutrition & physiology*. NY, USA: Nova Science Publishers, Inc. p. 210-24
- Scalbert A, Morand C, Manach C, Remesy C. (2002). Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed Pharmacother* 56:276-82
- Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, et al. (2002). The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc Natl Acad Sci USA* 99:14422-7.
- Scheppach W, Fabian C, Sachsm, Kasperh, . (1988). The effect of starch malabsorption on fecal short chain fatty acid excretion in man. *Scandinavian Journal of Gastroenterology* 23, 755-759
- Sela DA, Chapman J, Adeuya A, Kim JH, Chen F, Whitehead TR, et al. (2008). The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proc Natl Acad Sci USA* 105:18964-9.
- Serra J, Villoria A, Azpiroz F, Lobo B, Santos J, Accarino A, Malagelada J. (2010). Impaired intestinal gas propulsion in manometrically proven dysmotility and in irritable bowel syndrome. *Neurogastroenterol Motil* 22:401-406.
- Sieuwerts S, De Bok F, Mols E, De Vos W, Van Hylckama Vlieg J. (2008). A simple and fast method for determining colony forming units. *Lett Appl Microbiol*, 47:275-278.
- Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G et al. (2008). *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 105:16731-16736.
- Soret R, Chevalier J, De Coppet P, Poupeau G, Derkinderen P, Segain J, Neunlist M. (2010). Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. *Gastroenterology* 138:1772-1782.

- Sybesma W, Burgess C, Starrenburg M, Sinderen Dv, Hugenholtz J.(2004). Multivitamin production in *Lactococcus lactis* using metabolic engineering. *Metab Eng*, 6:109-115.
- Thibault R, Blachier F, Darcy-Vrillon B, de Coppet P, Bourreille A, Segain J.(2010). Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: a transport deficiency. *Inflamm Bowel Dis* 16:684-695.
- Tlaskalová-Hogenová H, Stepánková R, Hudcovic T, Tucková L, Cukrowska B, Lodinová-Zádníková R, et al.(2004). Comensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol Lett* 93:97-108
- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI.(2007). The human microbiome project. *Nature* 449:804-10
- Tzonis X, Vulevic J, Kuhnle GG, George T, Leonczak J, Gibson GR, et al. (2008). Flavanol monomer-induced changes to the human faecal microflora. *Br J Nutr* 99:782-92.
- van Summeren MJ, Braam LA, Lilien MR, Schurgers LJ, Kuis W, Vermeer C.(2009). The effect of menaquinone-7 (vitamin K2) supplementation on osteocalcin carboxylation in healthy prepubertal children. *Br J Nutr* 102:1171-1178.
- Videla S, Vilaseca J, Antolin M, Garcia-Lafuente A, Guarner F, Crespo E, et al.(2001). Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. *Am J Gastroenterol* 96:1486-93
- Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, Brown D, Stares MD, Scott P, Bergerat A et al.(2010). Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* doi: 10.1038/ismej.2010.118.
- Wall R, Ross R, Shanahan F, O'Mahony L, O'Mahony C, Coakley M, Hart O, Lawlor P, Quigley E, Kiely B et al.(2009). Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues. *Am J Clin Nutr* 89:1393-1401.
- Watzl B, Gierbach S, Roller M.(2005). Inulin, oligofructose and immunomodulation. *Br J Nutr* 93:S49-55.
- Welters CF, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG.(2002). Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. *Dis Colon Rectum* 45:621-7.
- Wilson ID, Nicholson JK. (2009). The role of gut microbiota in drug response. *Curr Pharm Des* 15:1519-23.
- Xu J, Mahowald MA, Ley RE, Lozupone CA, Hamady M, Martens EC, et al. (2007). Evolution of symbiotic bacteria in the distal human intestine. *PLoS Biol* 19:e156.

- Yuan J, Wang J, Liu X.(2007). Metabolism of dietary soy isoflavones to equol by human intestinal microflora; implications for health. *Mol Nutr Food Res* 51:765–81.
- Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, Mao Y, Zhang X, Pang X, Wei C et al.(2010). Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J* 4:232-241.
- Zoetendal EG, Akkermans AD, De Vos WM.(1998). Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* ;64:3854–9

RESEARCH PAPER I. IMPACT OF KAMUT® KHORASAN ON GUT MICROBIOTA AND METABOLOME IN HEALTHY VOLUNTEERS

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1. Introduction

Recent years are witnessing an increasing consumption of whole grains over refined ones, as recommended by countries and organizations around the globe (Council, 2012). Whole grains are concentrated sources of nutrients and phytochemicals, with well-known beneficial effects for human health (Jonnalagadda et al., 2011). Among these, a considerable body of epidemiological work has indicated that the intake of whole grain-based foods is inversely associated with the risk of developing chronic diseases, such as heart disease, type 2 diabetes and obesity (Giugliano and Esposito, 2008). In this respect, bacterial fermentation of the otherwise indigestible constituents of whole grains in the gastrointestinal tract has been suggested as partly responsible for the reduction of inflammatory markers (North et al., 2009).

There is ample evidence that diet can modulate both composition and functionality of the human gut microbiota (Louis et al., 2007; Candela et al., 2010a, 2012a; Cotillard et al., 2013), in a complex and dynamic interplay crucial for maintaining the host-microbiota mutualism (Cani and Delzenne, 2007; Candela et al., 2013). Escaping digestion in the small bowel, dietary fibers and prebiotics undergo fermentation in the colon, selectively stimulating the growth and/or activity of specific lower gut microbes, and resulting in the release of short chain fatty acids (SCFA), mainly acetate, propionate and butyrate (Gibson et al., 2004; Roberfroid, 2007; Flint et al., 2008). These major fermentation by-products are increasingly perceived to play a pivotal role at both colonic and systemic level, positively influencing several aspects of the host physiology (Neish, 2009; Kau et al., 2011).

The high carotenoid content (Abdel-Aal et al., 2007) and low allergenic potential (Simonato et al., 2002) make Khorasan wheat (Kamut), an ancient grain, very interesting. It has recently been shown that Kamut bread protects rats from oxidative stress to a greater extent than that afforded by whole-grain durum wheat (Benedetti et al., 2012) and that the fermentation process influences the ability of bread to provide this protection (Gianotti et al., 2011). Moreover, Kamut has been

demonstrated to promote the growth of probiotic *Lactobacillus* and *Bifidobacterium* strains (Marotti et al., 2012). Very recently, Sofi et al. (2013) confirmed in 22 healthy volunteers that a replacement diet with Kamut-based products could be effective in reducing markers of both oxidative stress and inflammatory status, as well as metabolic risk factors.

Although the effects of whole grains on the human gut microbiota appeared evident few years ago in healthy volunteers, in terms of increase in the fecal abundances of bifidobacteria and lactobacilli (Costabile et al., 2008), the information on how specific whole-grain cereals affect the intestinal microbial ecosystem is currently very poor and mainly derived from animal models (Neyrinck et al., 2012; Walter et al., 2013), and no reports exist describing the influence of Kamut on the microbial ecology of the human gut.

The aim of the present study was to explore the impact of Kamut® Khorasan on the gut microbiota and metabolic profiles in healthy volunteers. The study was carried out in 30 subjects randomly divided into two groups and administered foods based on two different cereals: Kamut® khorasan and wheat whole-grains. The fecal microbiota was characterized by means of the phylogenetic microarray platform High Taxonomic Fingerprint (HTF)-Microbi.Array (Candela et al., 2010b, 2012b). Metabolomic shifts in feces and urine were investigated using solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS).

2. Materials and methods

2.1 Participants and study design

A total of 30 adults aged between 25 to 53 years (mean, 37.0 ± 7.3), including 4 males and 26 females, were recruited into this study from the Ancona area, Italy. All subjects were healthy and had not received antibiotics and/or probiotics/prebiotics for at least 3 months prior to sampling. The participants were randomized to a Kamut® Khorasan (n = 15) or a whole wheat (n = 15) diet for 3 months. The study protocol was approved by the Ethics Committee of Azienda Ospedaliera

Universitaria Ospedali Riuniti Umberto Lancisi e Salesi di Ancona (protocol no. 212267). Written informed consent was obtained from all participants.

2.2 Dietary food composition and sample collection

All subjects followed a nutritionally balanced but not strictly defined diet that differed excepting for the contribution of two different cereal-based foods (baked goods and pasta) whose composition is reported in Table 1 according to Simonetti et al. (2013, submitted to European Journal of Nutrition).

Each participant was asked to collect a fecal and urine sample before entering the study (T0) and after 3-month dietary intervention (TF). Samples were immediately frozen at -80°C.

2.3 DNA extraction and PCR

Total microbial DNA was extracted from fecal samples as previously described (Candela et al., 2010b). DNA concentration and quality was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop® Technologies, Wilmington, DE, USA). Due to insufficient DNA content, 5 subjects were excluded from further analysis. PCR amplifications of the 16S rDNA gene were carried out in a Biometra Thermal Cycler T Gradient (Biometra, Göttingen, Germany) using the universal primer set 27F/1492r (Candela et al., 2010b). PCR products were purified with the High Pure PCR Cleanup Micro kit (Roche, Mannheim, Germany), eluted in 30 µL of sterile water and quantified with NanoDrop ND-1000.

2.4 HTF-Microbi.Array analysis and data processing

The phylogenetic structure of fecal samples was characterized using the ligase detection reaction-universal array (LDR-UA) platform High Taxonomic Fingerprint (HTF)-Microbi.Array, which allows the detection and quantification of 31 intestinal bacterial groups, covering up to 95% of the human gut microbiota (Candela et al., 2010b, 2012b). Slide chemical treatment, array production, LDR protocol and hybridization conditions were as in Centanni et al. (2013). All arrays

were scanned and processed according to the protocol and parameters already described by Candela et al. (2010b).

2.5 Solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) analysis

Preconditioning, absorption and desorption phases of SPME-GC analysis were performed according to Di Cagno et al. (2011).

All GC-MS raw files were converted to netCDF format via Chemstation (Agilent Technologies, Palo Alto, CA, USA) and subsequently processed with the XCMS toolbox (<http://metlin.scripps.edu/download/>) for automatic and simultaneous retention time alignment, matched filtration, peak detection, and peak matching. The resulting table containing information such as peak index (retention time-m/z pair) and normalized peak area was exported into R 3.0.0 (www.r-project.org) for subsequent statistical analysis (Serrazanetti et al., 2011).

Identification of molecules was carried out by comparing their retention times with those of pure compounds (Sigma-Aldrich, Milan, Italy), and confirmed by searching mass spectra in the available databases (NIST version 2005 and Wiley version 1996) and literature. Quantitative data of the identified compounds were obtained by interpolation of the relative area versus the internal standard area (Serrazanetti et al., 2011).

2.7 Statistics

Principal Component Analysis (PCA) and hierarchical clustering of the HTF-Microbi.Array data were performed using the R packages MADE4 (Culhane et al., 2005) and vegan (Oksanen et al., 2013). The temporal and inter-individual similarity of the microbiota profiles was assessed by Pearson's correlation coefficient. The significance of the differences in the microbiota composition in response to diet and between diet groups was estimated with Mann-Whitney U test, followed by adjustment for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) correction (Benjamini and Hochberg, 1995). $P < 0.05$ was considered statistically significant.

Canonical analysis of principal coordinates (CAP) was performed in the R environment using the vegan package (Oksanen et al., 2013) to discriminate between and within the diet groups based on metabolomic data.

Wiggum plot was designed as previously shown by Claesson et al. (2012). Briefly, the Kendall correlations among taxon abundance, urine and fecal metabolites were clustered by Pearson's correlation coefficient and Ward linkage in order to define bacterial co-abundance groups (CAGs) in the dataset. All correlations and all CAGs were controlled for multiple testing or permutational MANOVA.

3. Results

3.1 Modulation of the fecal microbiota by whole-grain intake

The fecal microbiota of 25 healthy volunteers was characterized before (T0) and after (TF) 3-month whole-grain dietary intervention by HTF-Microbi.Array (Fig. S1). The microbiota profiles were largely dominated by Bacteroidetes and Firmicutes, which generally accounted for >90% of the total microbial community. Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia were regularly present as minor components, with a mean relative abundance ranging from <1 to 4%.

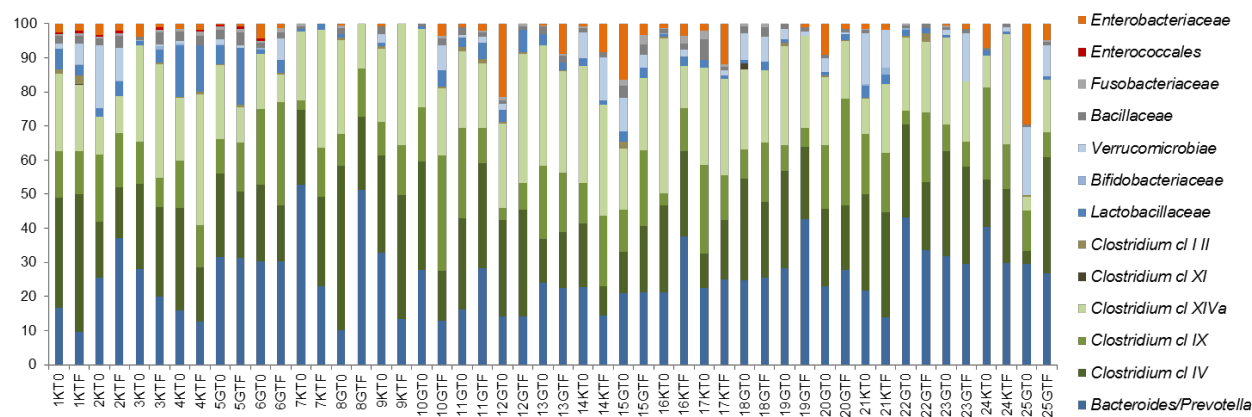


Fig. S1. High taxonomic level fingerprint of the fecal microbiota from 25 healthy human subjects before (T0) and after (TF) 3-month dietary intervention with Kamut® Khorasan (K, 11 subjects) or

whole wheat (G, 14 subjects). The relative abundance of each microbial family was determined using the HTF-Microbi.Array platform.

No significant difference in the microbiota composition was observed between and within dietary groups over time. Consistent with this, PCA and hierarchical clustering of the fecal microbiota patterns showed no segregation of the samples according to either diet group or pre- and post-intervention samples (Fig. S2). For most of the participants, the microbiota fingerprintings at T0 and TF were closely related, indicating a high individual specificity and temporal stability despite the change in diet (Fig. S2B). Stressing the individuality in the gut microbiota composition and in the response to dietary intervention, Pearson's correlation coefficients within subjects were >0.85 whereas the average inter-subject microbiota correlation was 0.77.

Even if none of the two whole-grain diets significantly altered the overall community structure of the fecal microbiota, a tendency towards a reduction in *Bacteroides/Prevotella* (Kamut, 21.5% vs whole wheat, 28.4%; FDR-adjusted $P = 0.22$) and an increase in members of *Clostridium* cluster XIVa (27.1% vs 19.9%; FDR-adjusted $P = 0.13$) was found following administration of Kamut compared to whole-wheat cereals (Fig. 1). Accordingly, the Bacteroidetes/Firmicutes ratio was lower in volunteers consuming Kamut with respect to the whole wheat-based diet group (mean \pm SEM, 0.33 ± 0.06 vs 0.46 ± 0.06).

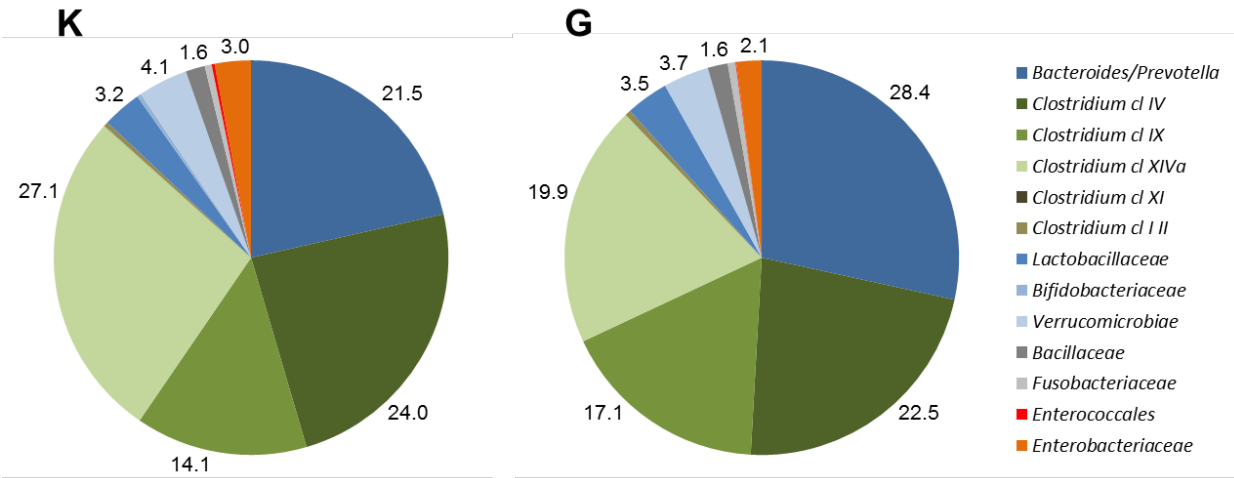
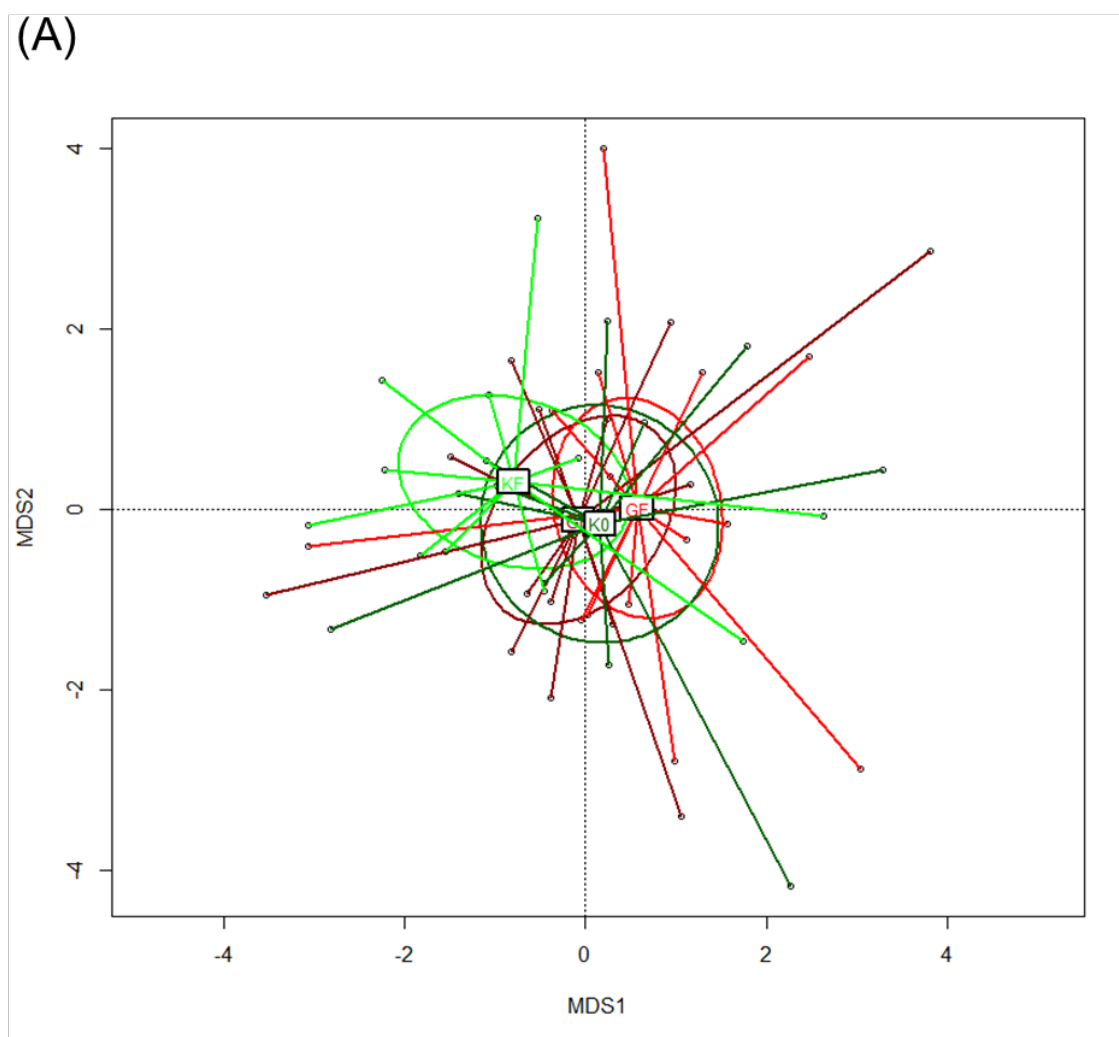


Fig. 1. Relative contribution of the major intestinal microbial families in the fecal microbiota of healthy human subjects after 3-month Kamut® Khorasan (K, 11 subjects)- or whole wheat (G, 14 subjects)-based diet. Mean values of relative abundance (%) for each microbial family were determined using the HTF-Microbi.Array platform. Only values greater than 1% are indicated.



(B)

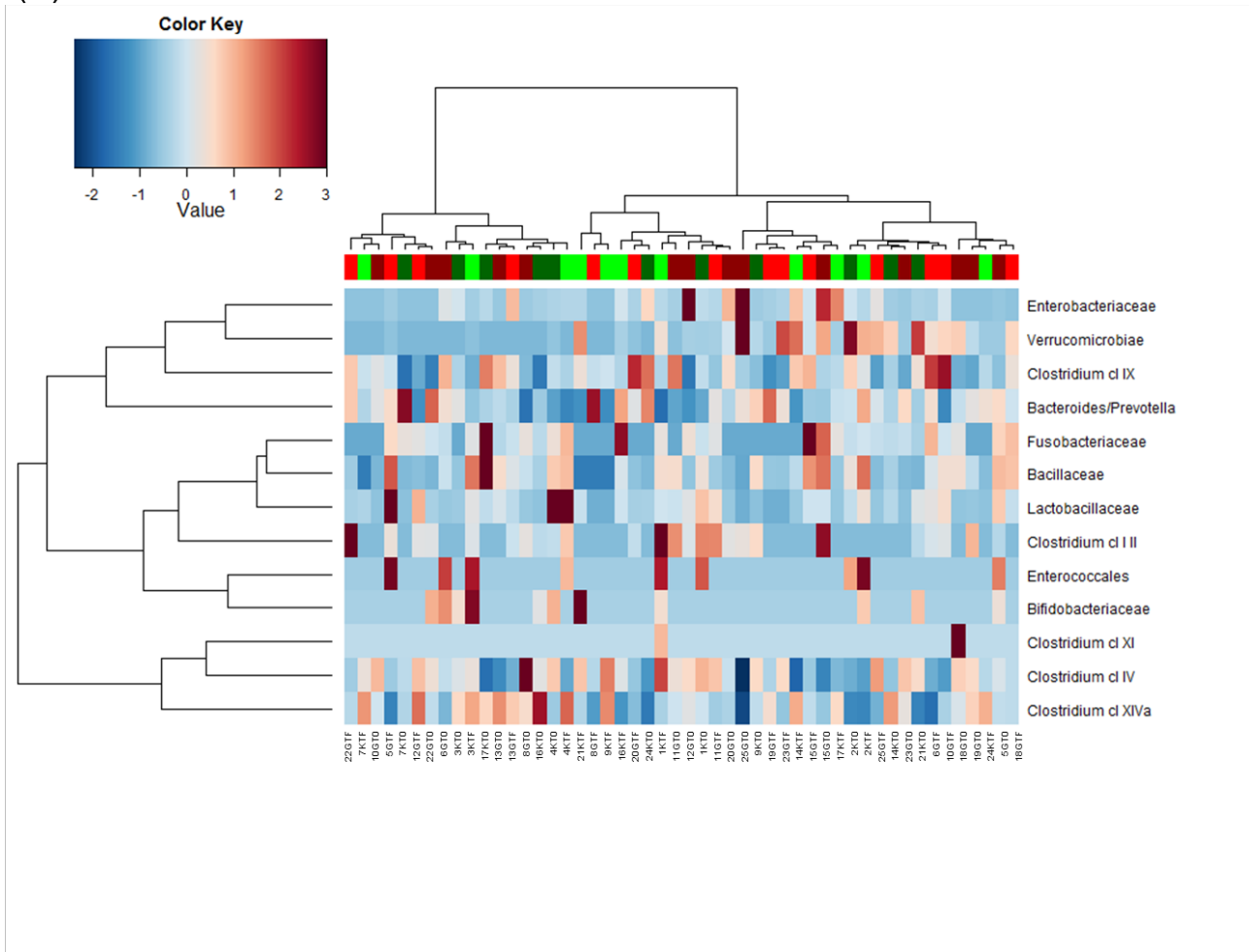


Fig. S2. Multivariate statistical analyses of the relative abundances of microbial families in the fecal microbiota from 25 healthy human subjects before and after 3-month dietary intervention with Kamut® Khorasan (11 subjects) or whole wheat (14 subjects). (A) PCA analysis. First and second ordination axes are plotted explaining 44.4 and 30.0% of the overall variance in the dataset, respectively. K0 (olive green) and KF (bright green), before and after Kamut® Khorasan-based diet; G0 (dark red) and GF (bright red), before and after whole wheat-based diet. (B) Hierarchical cluster analysis using Spearman's rank correlation with Ward's linkage. Subjects are clustered in the top dendrogram and color-coded as in (A).

3.2 Volatile compounds in feces and urine

Between 90 and 150 molecules belonging to the families of sulfur compounds, nitrogen compounds, aldehydes, ketones, esters, alcohols, phenols, organic acids, and hydrocarbons were detected in each sample of feces and urine. In order to identify and discriminate between and within the two whole grain-based diet groups, metabolic profile dataset was subjected to Canonical discriminant Analysis of Principal coordinates (CAP). In fact, CAP analysis demonstrated to allow a separation of stool samples collected before and after a dietary intervention period (Vitali et al., 2010).

In general, a high inter-individual variability was observed, thus hampering the evaluation of the change due to the diet. Concerning the fecal metabolome, the molecules released after 3-month whole wheat-based diet were mainly alcohols (isopropyl alcohol, 1-heptadecanol, oleyl alcohol, 2-butanol), sulfuric compounds (dimethyl trisulfide), acetone and SCFA (butanoic acid, 2-methyl butanoic acid, hexanoic acid and 3-methyl butanoic acid). Among these, short and medium chain fatty acids of butanoic acid were the most significant molecules able to discriminate T0 and TF samples (Fig. 2A).

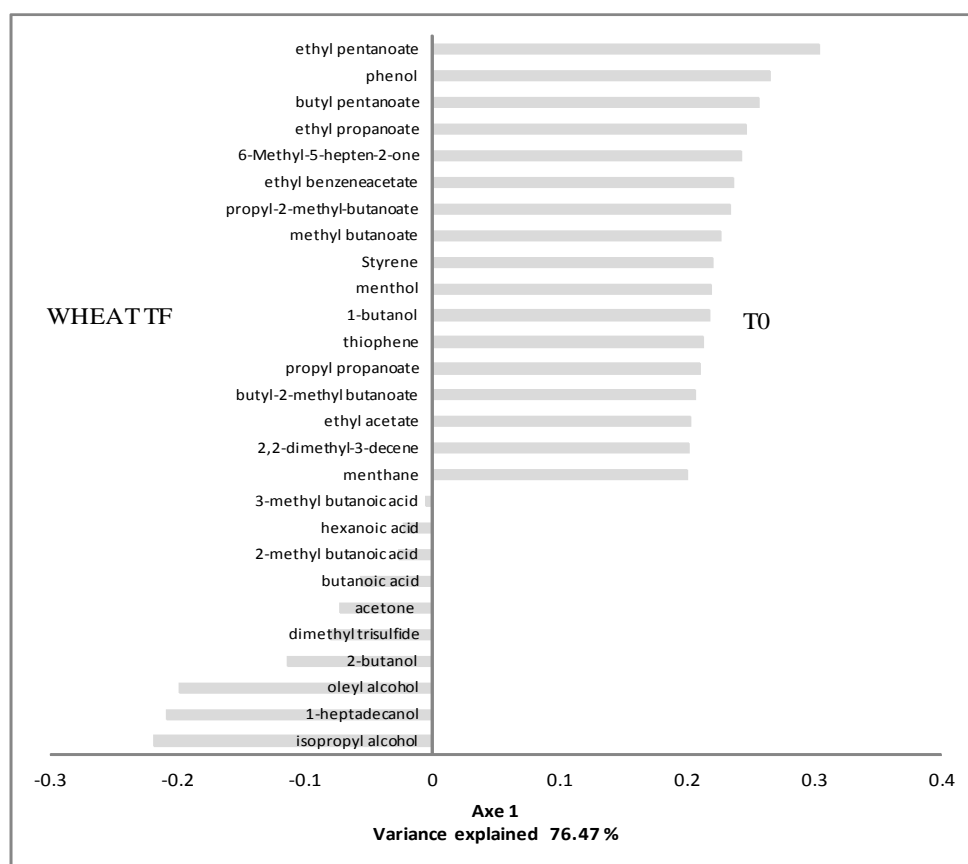


Fig. 2.A Loading coefficient plots of the fecal volatile organic compounds before (T0) and after (TF) 3-month whole wheat-based diet.

With regard to the Kamut-based diet group, some of the fecal metabolites identified were shared with the whole-wheat consumers, while other properly discriminated the consumption of Kamut as the main cereal in the diet. In particular, the molecules associated with the Kamut intake were ethyl-1-methyl-pentanoate, butanoic acid, dimethyl trisulfide, 1-tridecanol, pentanoic acid, hexanoic acid, 2,3-butanedione, 2-methyl butanoic acid, 3-methyl butanoic acid, isopropyl alcohol, propanoic acid, acetic acid, 2-butanol, and some compounds belonging to the group of terpenes (Fig. 2B). As for the whole wheat-based diet group, short and medium chain fatty acids, both linear and branched, better discriminated the volatile profiles before and after Kamut consumption.

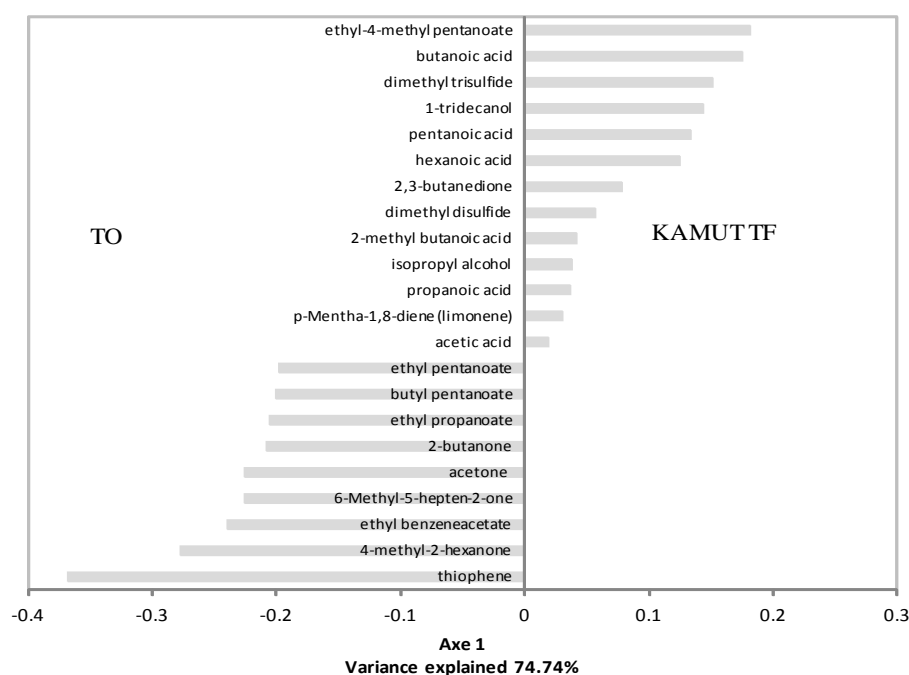


Fig. 2.B Loading coefficient plots of the fecal volatile organic compounds before (T0) and after (TF) 3-month whole Kamut® Khorasan (B)-based diet.

Figure 2C shows the comparison of the fecal metabolic patterns between the two dietary interventions. The most significant molecules in whole-wheat consumers able to differentiate the diet groups were acetone, ethyl-3-methyl butanoate, ethyl 4-octane, thiophene, 1-heptadecanol, 4-methyl-2-hexanone, oleyl alcohol, isopropyl alcohol, n-hexyl butanoate, trichloromethane, and ethyl-2-methylbutanoate. For Kamut consumers, the molecules better discriminating between diets were 2-nonanol, phenol, 4-methylpentanoic acid, 1-tridecanol, tetradecanal, 2,2-dimethyl-3-decene, methyl butanoate, n-hexyl-2-methyl butyrate, pentanoic acid, heptyl-4-methyl pentanoate, 1-pentanol, propyl butanoate and other, such as terpenes, short and medium chain fatty acids and esters. Both whole-grain diets were characterized by esters of butyric acid.

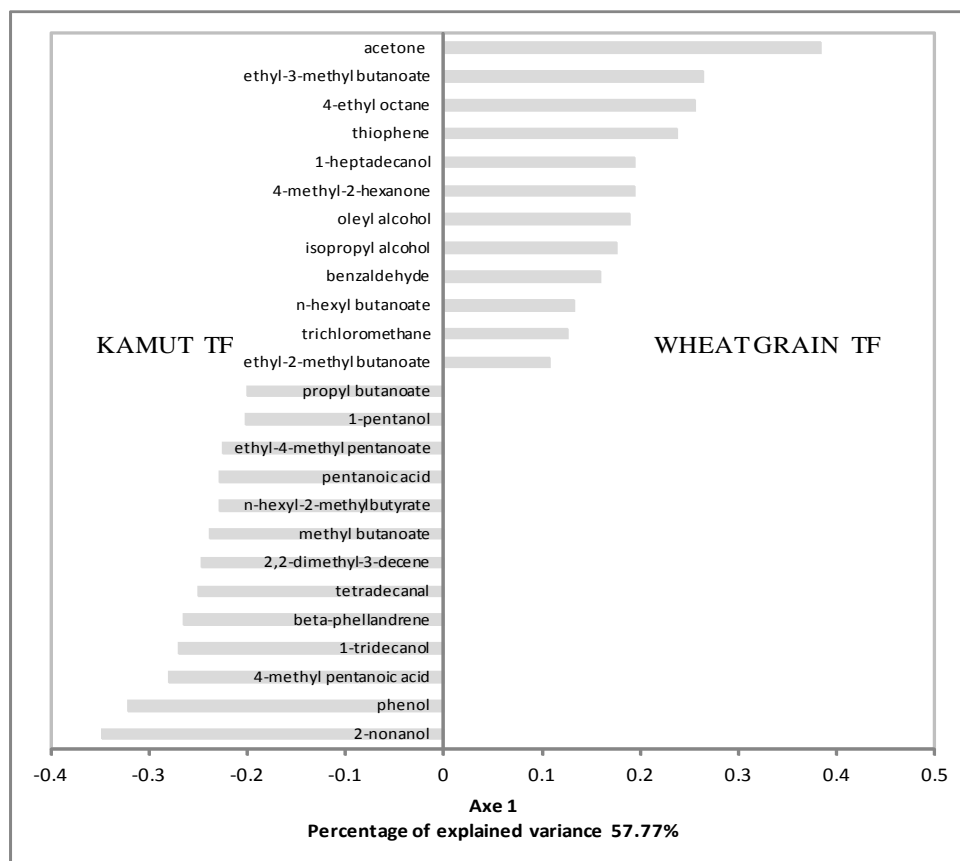


Fig. 2.C Loading coefficient plots of the fecal volatile organic compounds of the comparison between the two dietary intervention groups. Data are representative of three independent experiments.

In terms of urinary metabolic profiles, the molecules differentiating the dietary groups were phenols, such as phenol 4-(1,1,3,3-tetramethylbutyl) and phenol-m-tert-butyl, ethanol, methyl-2-thienylacetate, 2-pentyl furan, 2 butanone, acetone and nonanal, and all of them derived from Kamut consumption (Fig. 3).

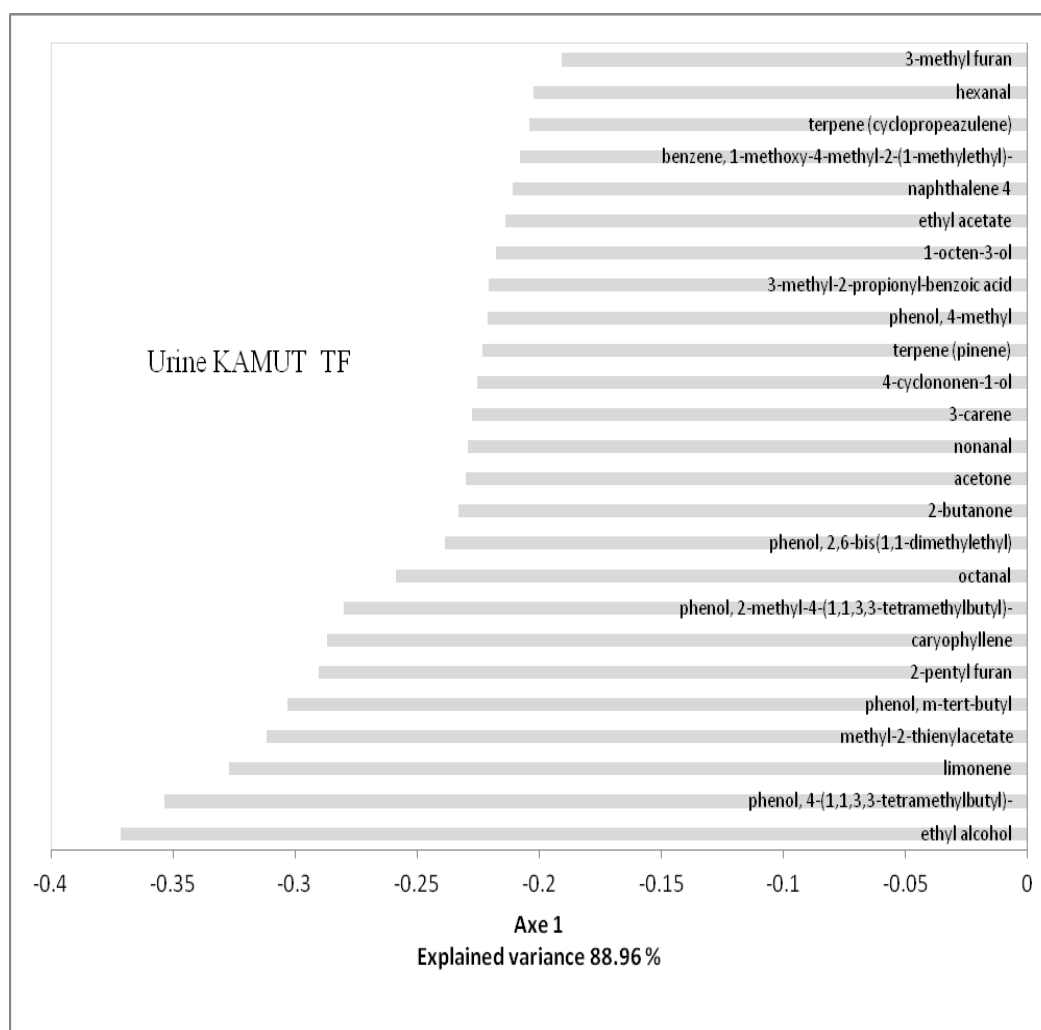


Fig. 3. Loading coefficient plot of the urinary volatile organic compounds after 3-month Kamut® Khorasan- or whole wheat-based diet. Data are representative of three independent experiments.

The small differences observed between the two dietary groups did not allow a significant discrimination of the diets on the basis of specific molecule shifts. The failure to identify specific markers in both diets could be related to the fact that the most important compound classes are made up by non-volatile compounds.

3.4 Meta-analysis highlights relationships among gut microbiota, fecal and urine metabolites

To typify patterns among gut microbiota, fecal and urine metabolites, we sought co-abundance associations in the overall dataset, by clustering correlated taxa and metabolites in co-abundance groups (CAGs). Two CAGs displaying significantly different inter-relationships from each other (P

<0.001), and describing different microbiota-metabotype inter-connections were identified: SCFA CAG and *Enterobacteriaceae* CAG. The relationships between the two groups are shown in the Wiggum plot of Fig. 4. According to our analysis, SCFA CAG was characterized by the dominance of *Clostridium* cluster IV and *Clostridium* cluster XIVa, and the concomitant presence of butyrate, propionate and acetate. *Enterobacteriaceae* CAG comprised, among others, *Clostridium* cluster IX and *Verrucomicrobiae*.

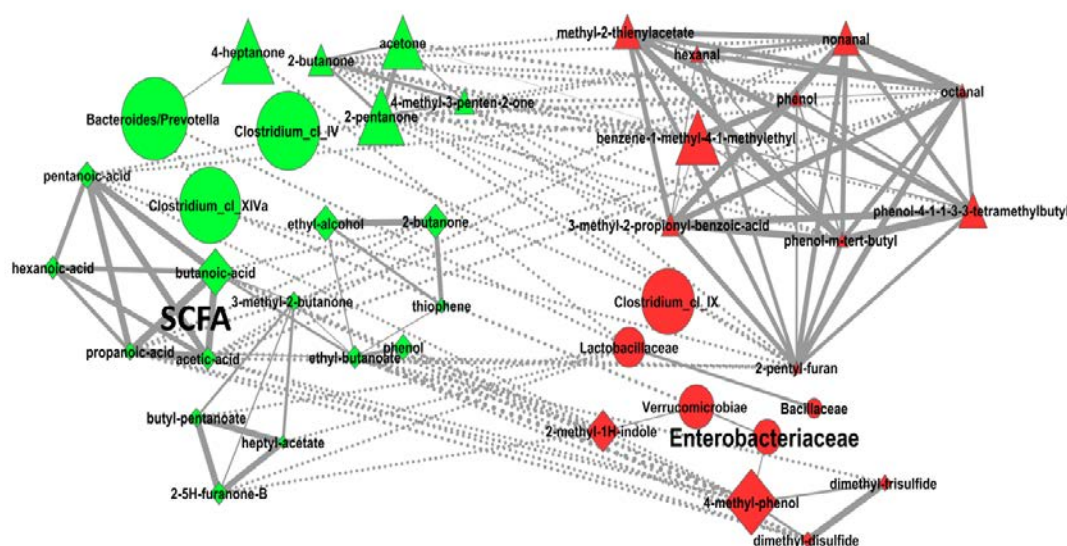


Fig. 4. Network plot showing correlation relationships among clusters of microbial groups, fecal and urine metabolites. Taxa, fecal and urine metabolites are shown respectively as circles, diamonds or triangles, and their size is proportional to the average abundance in the study. Each node is color-coded for co-abundance group (CAG) and each line highlights a significant correlation between two CAG members. Solid lines indicate positive correlation, whereas dot lines indicate negative correlation. Thickness of the lines is proportional to correlation strength.

Interestingly, after 3-month Kamut consumption, the gut microbiota-metabolome fingerprint showed a higher level of SCFA CAG with respect to whole-wheat consumers. In particular 16/23 members of SCFA CAG, including butanoic acid, and *Clostridium* clusters IV and XIVa, were more abundant in the Kamut-based diet group, compared to the whole-wheat one. Conversely, Kamut had not impact on *Enterobacteriaceae* CAG, which remained stable during the study. To the other end, the consumption of whole wheat led to a rearrangement in several components of *Enterobacteriaceae* CAG. Indeed, we observed an increase in *Clostridium* cluster IX, *Lactobacillaceae*, methyl-phenol and methyl-2-thienylacetate, and a concomitant decrease in *Enterobacteriaceae*, dimethyl-trisulfide, phenol and 2-pentyl-furan.

4. Discussion

Daily consumption of whole grain wheat exerts a prebiotic effect on the human gut microbiota. This prebiotic activity together with antioxidant activity may contribute to the protective health effects of whole grain wheat (Costabile et al., 2008). In particular, fiber, composed of plant structural and storage polysaccharides, and plant secondary polyphenolic compounds are thought to contribute significantly to the underlying protective mechanisms (Tuohy et al., 2009). Both of them potentially interact with the gut microbiota: microbial transformation modifies bioavailability and activity of many polyphenols, and fiber represents the major carbohydrate substrate for colonic fermentation. However, up to date, there is little information on whether or how the type of cereal specifically affects the gut microbiota and furthermore, the impact of Kamut® Khorasan on the gut microbial ecology has never been explored.

In the present work, we analyzed the effect of 3-month intake of Kamut vs whole wheat on the stability and composition of the fecal microbiota, and of fecal and urine metabolites, and the overall associations between microbiota and metabolic profiles. Consistent with previous dietary intervention studies (Vitali et al., 2010; Lappi et al., 2013; Rampelli et al., 2013), our high

taxonomic-level analysis showed that the microbiota composition and stability did not significantly differ between the diet groups. However, the gut microbiota of Kamut consumers tended to be enriched in members of *Clostridium* cluster XIVa and depleted in *Bacteroides/Prevotella*. Interestingly, the clostridial clusters IV and XIVa contain the main carbohydrate-utilizing butyrate producers in the human gut (Louis et al., 2007), and their relative proportions have previously been demonstrated to increase as a result of whole grain wheat feeding (Conlon et al., 2012; Lappi et al., 2013). Conversely, a *Bacteroides*-dominated microbiota has been positively associated with the intake of fat (Wu et al., 2011), and *Bacteroides* spp. have been reported as more abundant in the colon of humans eating meat compared to vegetarians (Zimmer et al., 2011). In terms of metabolic patterns, both whole-grain diets were characterized by short and medium chain fatty acids, even if butyrate and its esters better discriminated the Kamut-based diet. SCFA and especially butyrate, have been shown to be pivotal in several host physiological aspects, such as nutrition, immune function, signaling, proliferation control and protection from pathogen colonization (Tremaroli and Bäckhed, 2012). In particular, butyrate has been shown to have an anti-inflammatory role and control oxidative stress in the colonic mucosa (Russo et al., 2012). Co-abundance analysis of microbiota and metabolome allowed us to hypothesize the presence of 1 health-promoting CAG (SCFA CAG) and 1 polyvalent CAG (*Enterobacteriaceae* CAG), including both normal commensal bacterial groups and pathobionts, and their associated metabolites. The first CAG, which was more abundant in the Kamut-based diet group, included microbial genera, such as *Roseburia*, *Faecalibacterium*, *Blautia*, *Dorea* and *Ruminococcus*, which are known producers of SCFA, as well as ethanol, hydrogen and carbon dioxide (Chassard and Lacroix, 2013). Also *Enterobacteriaceae* CAG comprised bacterial groups known to produce SCFA, such as *Clostridium* cluster IX and *Verrucomicrobiae*, but these microorganisms generally produce lower SCFA amounts compared to the members of SCFA CAG (Chassard and Lacroix, 2013). Furthermore,

Enterobacteriaceae CAG included pro-inflammatory bacteria, which could negatively impact on the health status of the human meta-organism.

Regardless of the rest of the diets, Kamut-based diet presented some compositional differences, such as protein content, total fiber (due to soluble fiber), polyphenolic compounds and other antioxidant compounds (folic acid and vitamin E), and selenium concentration (Table 1). In particular, Kamut-based cereal foods accounted for a high protein content, which implies a different availability of substrate for the intestinal microbiota. In fact, Gibson et al. (2008) reported that between 3 and 25 g of proteins and peptides enter the large bowel every day from the diet as well as from endogenous sources, such as host tissue, pancreatic enzymes and other secretions. In the large gut these substances are depolymerized in short peptides and amino acids by a mixture of residual pancreatic endopeptidases and bacterial proteases and peptidases. This material becomes a substrate for the gut microbiota, resulting eventually in the production of SCFA by reductive deamination (Blachier et al., 2007), but also phenolic and indolic compounds, as well as toxic amines and ammonia through the metabolism of aromatic amino acids. In this regard, the higher abundance of both SCFA and phenol compounds detected in the Kamut-based diet group may be related to the degradation of proteins as an energy source (Blaut and Clavel, 2007), and suggest a higher concentration of aromatic amino acids in the colon of Kamut consumers.

The higher concentration of polyphenolic substances present in Kamut flour and products has been already correlated to the protection from oxidative stress (Gianotti et al., 2011). However, data on the impact of polyphenols on the gut microbiota are scarce. Dietary polyphenols unabsorbed in the small intestine reach the colon where they undergo substantial structural modifications. In fact, the colonic microbiota hydrolyzes glycosides into aglycones and degrades them to simple phenolic acids (Aura et al., 2005). These polyphenol catabolites are then available to exert their biological activities systemically within the host (Tuohy et al., 2012). The health benefits from phenolic consumption should be attributed to both their bioactive metabolites and the modulation of the

intestinal bacterial population (Lee et al., 2006). In particular, *Clostridium* and *Eubacterium* genera have been reported to be involved in the metabolism of several phenolic compounds (Selma et al., 2009). The increase in the relative abundance of *Clostridium* cluster XIVa and phenol compounds specifically related to the Kamut-based dietary intervention as observed in our study, seem to partially confirm the findings of Selma et al. (2009), who also reported how different metabolic pathways of polyphenol degradation converge to phenol compounds.

Finally, the higher intake of selenium by Kamut-based foods could be associated to the biological effects of Se incorporation into selenoproteins, and selenoproteins that are involved in the activation, proliferation, and differentiation of cells driving innate and adaptive immune responses. Dietary Se and selenoproteins are not only important for initiating or enhancing immunity, but they are also involved in immunoregulation, which is crucial for preventing excessive responses that may lead to autoimmunity or chronic inflammation (Huang et al., 20012). A recent study demonstrated an interesting effect of Se status on the composition of gut microbiota in mice by 454-pyrosequencing (Kasaikina et al., 2011). The authors evidenced an increased diversity in the intestinal microbiota, and in particular, an opposite correlation of dietary Se supplementation with the genus *Parabacteroides* of the phylum Bacteroidetes. A modest increase within clostridia was also shown. Although pyrosequencing and microarray approaches are only partially comparable, the findings of Kasaikina et al. (2011) are somehow similar to the trends observed in our study.

5. Conclusion

To our knowledge, this is the first report describing the effect of Kamut on the gut microbial ecology. According to our results, the introduction of whole grains in the human diet had a strong impact on volatile metabolites. In particular, phenol, nonanol and SCFA, mainly butyrate and its esters, appeared to have a particular role in discriminating the diet of Kamut. On the other hand, the whole wheat was better characterized by the release of alcohols, such as oleyl alcohol and isopropyl

alcohol. With regard to the gut microbiota, a generally less pronounced effect was observed, with a 24.5% reduction in *Bacteroides/Prevotella* and a 36.3% enrichment in *Clostridium* cluster XIVa after Kamut intake compared to whole wheat. The co-abundance analysis showed a higher representation of a potentially health-promoting CAG, including SCFA as well as butyrate-producing members of the gut microbiota with anti-inflammatory properties, for the Kamut-based diet group.

Together with the high concentration of Se in Kamut-based foods, our results may represent one of the factors supporting our previous *in vivo* findings related to the anti-inflammatory activity and counteraction of oxidative stress by Kamut-based cereal foods. Further work is needed to deeply explore the effects of Kamut and its components on the gut microbiota and metabolome, as well as the pervasive microbiota-host physiological interconnections.

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References

- Abdel-Aal el, S. M., Young, J. C., Rabalski, I., Hucl, P., & Fregeau-Reid, J. (2007). Identification and quantification of seed carotenoids in selected wheat species. *Journal of Agricultural and Food Chemistry*, 55, 787-794.
- Aura, A. M., Martin-Lopez, P., O'Leary, K. A., Williamson, G., Oksman-Caldentey, K. M., Poutanen, K., & Santos-Buelga, C. (2005). *In vitro* metabolism of anthocyanins by human gut microflora. *European Journal of Nutrition*, 44, 133-142.
- Benedetti, S., Primiterra, M., Tagliamonte, M. C., Carnevali, A., Gianotti, A., Bordoni, A., & Canestrari, F. (2012). Counteraction of oxidative damage in the rat liver by an ancient grain (Kamut brand khorasan wheat). *Nutrition*, 28, 436-441.

- Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, 57, 289-300.
- Blachier, F., Mariotti, F., Huneau, J. F., & Tome, D. (2007). Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. *Amino Acids*, 33, 547-562.
- Blaut, M., & Clavel, T. (2007). Metabolic diversity of the intestinal microbiota: implications for health and disease. *Journal of Nutrition*, 137, 751S-755S.
- Candela, M., Biagi, E., Turrone, S., Maccaferri, S., Figini, P., & Brigidi, P. (2013). Dynamic efficiency of the human intestinal microbiota. *Critical Reviews in Microbiology*. In press.
- Candela, M., Biagi, E., Maccaferri, S., Turrone, S. & Brigidi, P. (2012a). Intestinal microbiota is a plastic factor responding to environmental changes. *Trends in Microbiology*, 20, 385-391.
- Candela, M., Rampelli, S., Turrone, S., Severgnini, M., Consolandi, C., De Bellis, G., Masetti, R., Ricci, G., Pession, A., & Brigidi, P. (2012b). Unbalance of intestinal microbiota in atopic children. *BMC Microbiology*, 12, 95.
- Candela, M., Maccaferri, S., Turrone, S., Carnevali, P., & Brigidi, P. (2010a). Functional intestinal microbiome, new frontiers in prebiotic design. *International Journal of Food Microbiology*, 140, 93-101.
- Candela, M., Consolandi, C., Severgnini, M., Biagi, E., Castiglioni, B., Vitali, B., De Bellis, G., & Brigidi, P. (2010b). High taxonomic level fingerprint of the human intestinal microbiota by ligase detection reaction--universal array approach. *BMC Microbiology*, 10, 116.
- Cani, P. D., & Delzenne, N. M. (2007). Gut microflora as a target for energy and metabolic homeostasis. *Current Opinion in Clinical Nutrition & Metabolic Care*, 10, 729-734.
- Centanni, M., Turrone, S., Biagi, E., Severgnini, M., Consolandi, C., Brigidi, P., & Candela, M. (2013). A novel combined approach based on HTF-Microbi.Array and qPCR for a reliable

- characterization of the *Bifidobacterium*-dominated gut microbiota of breast-fed infants. *FEMS Microbiology Letters*, 343, 121-126.
- Chassard, C., & Lacroix, C. (2013). Carbohydrates and the human gut microbiota. *Current Opinion in Clinical Nutrition & Metabolic Care*, 16, 453-460.
- Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G. F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., Stanton, C., Marchesi, J. R., Fitzgerald, A. P., Shanahan, F., Hill, C., Ross, R. P., & O'Toole, P. W. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature*, 488, 178-184.
- Conlon, M. A., Kerr, C. A., McSweeney, C. S., Dunne, R. A., Shaw, J. M., Kang, S., Bird, A. R., Morell, M. K., Lockett, T. J., Molloy, P. L., Regina, A., Toden, S., Clarke, J. M., & Topping, D. L. (2012). Resistant starches protect against colonic DNA damage and alter microbiota and gene expression in rats fed a Western diet. *Journal of Nutrition*, 142, 832-840.
- Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., Gibson, G. R., & Tuohy, K. M. (2008). Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. *British Journal of Nutrition*, 99, 110-120.
- Cotillard, A., Kennedy, S. P., Kong, L. C., Prifti, E., Pons, N., Le Chatelier, E., Almeida, M., Quinquis, B., Levenez, F., Galleron, N., Gougis, S., Rizkalla, S., Batto, J. M., Renault, P., ANR MicroObes consortium, Doré, J., Zucker, J. D., Clément, K., Ehrlich, S. D., Blottière, H., Leclerc, M., Juste, C., de Wouters, T., Lepage, P., Fouqueray, C., Basdevant, A., Henegar, C., Godard, C., Fondacci, M., Rohia, A., Hajduch, F., Weissenbach, J., Pelletier, E., Le Paslier, D., Gauchi, J. P., Gibrat, J. F., Loux, V., Carré, W., Maguin, E., van de Guchte, M., Jamet, A.,

- Boumezbeur, F., & Layec, S. (2013). Dietary intervention impact on gut microbial gene richness. *Nature*, *500*, 585-588.
- Council, W. G. (2012). Whole grain guidelines worldwide.
<http://www.wholegrainscouncil.org/whole-grains-101/whole-grain-guidelines-worldwide>.
- Culhane, A. C., Thioulouse, J., Perrière, G., & Higgins, D. G. (2005). MADE4: an R package for multivariate analysis of gene expression data. *Bioinformatics*, *21*, 2789-2790.
- Di Cagno, R., De Angelis, M., De Pasquale, I., Ndagijimana, M., Vernocchi, P., Ricciuti, P., Gagliardi, F., Laghi, L., Crecchio, C., Guerzoni, M. E., Gobbetti, M., & Francavilla, R. (2011). Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization. *BMC Microbiology*, *11*, 219.
- Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R., & White, B. A. (2008). Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nature Reviews Microbiology*, *6*, 121-131.
- Gianotti, A., Danesi, F., Verardo, V., Serrazanetti, D. I., Valli, V., Russo, A., Riciputi, Y., Tossani, N., Caboni, M. F., Guerzoni, M. E., & Bordoni, A. (2011). Role of cereal type and processing in whole grain in vivo protection from oxidative stress. *Frontiers in Bioscience (Landmark Ed)*, *16*, 1609-1618.
- Gibson, G. R., Probert, H. M., Loo, J. V., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, *17*, 259-275.
- Gibson, G. R., Macfarlane, G. T., & Cummings, J. H. (1988). Occurrence of sulphate reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. *Journal of Applied Bacteriology*, *65*, 103-111.
- Giugliano, D., & Esposito, K. (2008). Mediterranean diet and metabolic diseases. *Current Opinion in Lipidology*, *19*, 63-68.

- Huang, Z., Rose, A. H., & Hoffmann, P. R. (2012). The role of selenium in inflammation and immunity: From molecular mechanisms to therapeutic opportunities. *Antioxidants & Redox Signaling*, *16*, 705-743.
- Jonnalagadda, S. S., Harnack, L., Liu, R. H., McKeown, N., Seal, C., Liu, S., & Fahey, G. C. (2011). Putting the whole grain puzzle together: health benefits associated with whole grains--summary of American Society for Nutrition 2010 Satellite Symposium. *The Journal of Nutrition*, *141*, 1011S-1022S.
- Kasaikina, M. V., Kravtsova, M. A., Lee, B. C., Seravalli, J., Peterson, D. A., Walter, J., Legge, R., Benson, A. K., Hatfield, D. L., & Gladyshev, V. N. (2011). Dietary selenium affects host selenoproteome expression by influencing the gut microbiota. *The FASEB Journal*, *25*, 2492-2499.
- Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., & Gordon, J. I. (2011). Human nutrition, the gut microbiome, and immune system: envisioning the future. *Nature*, *474*, 327-336.
- Lappi, J., Salojärvi, J., Kolehmainen, M., Mykkänen, H., Poutanen, K., de Vos, W. M., & Salonen, A. (2013). Intake of whole-grain and fiber-rich rye bread versus refined wheat bread does not differentiate intestinal microbiota composition in Finnish adults with metabolic syndrome. *Journal of Nutrition*, *143*, 648-655.
- Lee, H. C., Jenner, A. M., Lowa, C. S., & Lee, Y. K. (2006). Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Research in Microbiology*, *157*, 876-884.
- Louis, P., Scott, K. P., Duncan, S. H., & Flint, H. J. (2007). Understanding the effects of diet on bacterial metabolism in the large intestine. *Journal of Applied Microbiology*, *102*, 1197-1208.
- Marotti, I., Bregola, V., Aloisio, I., Di Gioia, D., Bosi, S., Di Silvestro, R., Quinn, R., & Dinelli, G. (2012). Prebiotic effect of soluble fibers from modern and old durum-type wheat varieties on

- Lactobacillus* and *Bifidobacterium* strains. *Journal of the Science of Food and Agriculture*, 92, 2133-2140.
- Neish, A. S. (2009). Microbes in gastrointestinal health and disease. *Gastroenterology*, 136, 65-80.
- Neyrinck, A. M., Possemiers, S., Verstraete, W., De Backer, F., Cani, P. D., & Delzenne, N. M. (2012). Dietary modulation of clostridial cluster XIVa gut bacteria (*Roseburia* spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. *The Journal of Nutritional Biochemistry*, 23, 51-59.
- North, C. J., Venter, C. S., & Jerling, J. C. (2009). The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. *European Journal of Clinical Nutrition*, 63, 921-933.
- Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). vegan: Community Ecology Package. R package version 2.0-7. <http://CRAN.R-project.org/package=vegan>.
- Rampelli, S., Candela, M., Severgnini, M., Biagi, E., Turrone, S., Roselli, M., Carnevali, P., Donini, L., & Brigidi, P. (2013). A probiotics-containing biscuit modulates the intestinal microbiota in the elderly. *The Journal of Nutrition Health and Aging*, 17, 166-172.
- Roberfroid, M. (2007). Prebiotics: the concept revisited. *The Journal of Nutrition*, 137, 830S-837S.
- Russo, I., Luciani, A., De Cicco, P., Troncone, E., & Ciacchi, C. (2012). Butyrate attenuates lipopolysaccharide-induced inflammation in intestinal cells and Crohn's mucosa through modulation of antioxidant defense machinery. *PLoS One*, 7, e32841.
- Selma, M. V., Espin, J. C., & Tomas-Barberan, F. A. (2009). Interaction between phenolics and gut microbiota: role in human health. *Journal of Agricultural and Food Chemistry*, 57, 6485-6501.
- Serrazanetti, D. I., Ndagijimana, M., Sado, S. L., Corsetti, A., Vogel, R. F., Ehrmann, M., & Guerzoni, M. E. (2011). Acid stress-mediated metabolic shift in *Lactobacillus sanfranciscensis* LSCE1. *Applied and Environmental Microbiology*, 77, 2656-2666.

- Simonato, B., Pasini, G., Giannattasio, M., & Curioni, A. (2002). Allergenic potential of Kamut wheat. *Allergy*, 57, 653-654.
- Simonetti, E., Trozzi, C., Gesuita, R., Raffaelli, F., & Mazzanti, L. (2013). Evaluation of antioxidative and diabetes-preventive properties of an ancient grain, Kamut® Khorasan wheat, in healthy volunteers. Submitted to *European Journal of Nutrition*.
- Slavin, J. (2003). Why whole grains are protective: biological mechanisms. *Proceedings Of The Nutrition Society*, 62, 129-134.
- Sofi, F., Whittaker, A., Cesari, F., Gori, A. M., Fiorillo, C., Becatti, M., Marotti, I., Dinelli, G., Casini, A., Abbate, R., Gensini, G. F., & Benedettelli, S. (2013). Characterization of Khorasan wheat (Kamut) and impact of a replacement diet on cardiovascular risk factors: cross-over dietary intervention study. [*European Journal of Clinical Nutrition*](#), 67, 190-195.
- Sofi, F., Ghiselli, L., Cesari, F., Gori, A. M., Mannini, L., Casini, A., Vazzana, C., Vecchio, V., Gensini, G. F., Abbate, R., & Benedettelli, S. (2010). Effects of short-term consumption of bread obtained by an old Italian grain variety on lipid, inflammatory, and haemorheological variables: an intervention study. *Journal of Medicinal Food*, 13, 1-6.
- Tremaroli, V., & Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature*, 489, 242-249.
- Tuohy, K. M., Conterno, L., & Gasperotti, M., Viola, R. (2012). Up-regulating the human intestinal microbiome using whole plant foods, polyphenols, and/or fiber. *Journal of Agricultural and Food Chemistry*, 60, 8776- 8782.
- Tuohy, K. M., Gougoulas, C., Shen, Q., Walton, G., Fava, F., & Ramnani, P. (2009). Studying the human gut microbiota in the trans-omics era – focus on metagenomics and metabonomics. *Current Pharmaceutical Design*, 15, 1415–1427.

- Vitali, B., Ndagijimana, M., Cruciani, F., Carnevali, P., Candela, M., Guerzoni, M. E., & Brigidi, P. (2010). Impact of a synbiotic food on the gut microbial ecology and metabolic profiles. *BMC Microbiology*, 10, 4.
- Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 334, 105-108.
- Zimmer, J., Lange, B., Frick, J. S., Sauer, H., Zimmermann, K., Schwiertz, A., Rusch, K., Klosterhalfen, S., & Enck, P. (2012). A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. *European Journal of Clinical Nutrition*, 66, 53-60.

CHAPTER II. USE OF FIE-MS AND GC-tof-MS TO STUDY THE EFFECT OF WHOLE GRAINS CONSUMPTION ON THE HUMAN METABOLOME

1. Introduction

Several studies shown that khorasan wheat, an ancient grain sold now as kamut® has some antioxidant properties, moreover it has a low allergenic potential (Simonato et al., 2002). In 2012, Benedetti et al. demonstrated that Kamut bread is more effective in protecting rats from oxidative stress than durum wheat and also that the fermentation process have an impact in the ability of bread to provide this protection (Gianotti et al., 2011). Sofi et al. (2013) evaluated the effect of kamut consumption in 22 healthy participants, and they hypothesized that a high intake diet of kamut derivatives might reduce markers of both oxidative stress and inflammatory status, as well as metabolic risk factors.

The lack of nutritional biomarkers that can predict functional outcomes and chronic diseases, as well as improvement of dietary assessment and planning methods (Institute of Medicine of the National Academies, 2007) is a difficult question for researchers. Analysis of dietary biomarkers can be expensive and invasive, so the need for non-invasive, inexpensive methods is essential (Thompson et al., 2010; Institute of Medicine of the National Academies, 2007). Food biomarkers or nutrient intake (or exposure) are able to objectively assess dietary intake/status without the bias of self-reported dietary intake errors (Hardin, 2009; McCabe-Sellers, 2010), and also overcome the problem of intra-individual diet variability (Monsen, 2003).

Food biomarkers are suitable for their ability to more precisely measure nutritional intake/status versus self-reported methods, validate self-reported intake measures, estimate intake of dietary components when food composition databases are inadequate, and to more accurately correlate dietary intake with disease risk and nutritional status (Potischman, 2003). Even though the analysis of dietary biomarkers usually give more accurate information, exogenous factors like genetic variability, lifestyle/physiologic factors (e.g., smoking), dietary factors (e.g., nutrient-nutrient interaction), biological sample and analytical methodology may bias the final result (Jenab et al., 2009).

Up to now, the number of recognized biomarkers is limited to a narrow range of foods and food components (Penn et al., 2010). Therefore, an exhaustive analysis of metabolites in biological samples using metabolomics approach can have a great positive effect on the discovery of dietary biomarkers (Lloyd et al., 2013; Rasmussen et al., 2012; Stella et al., 2006). Several studies demonstrated that flow infusion electrospray-ionization mass spectrometry (FIE-MS) (Jenab et al., 2009; Mennen et al., 2006) and nuclear magnetic resonance spectrometry (Favé et al., 2009, Scalbert et al., 2009) are effective for metabolomics analysis.

Some studies reported that plasma alkylresorcinol (AR) concentrations can be considered as whole grain wheat/rye biomarkers. In 2007, Linko-Parvinen et al., observed that total plasma AR augments with whole grains intake and reduces with refined bread intake after one week. Others metabolites correlated to whole grain intake are: 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl) propanoic acid (DHPPA) which are excreted through urine (Aubertin-Leheudre et al., 2008). DHBA and DHPPA were both reported to be significantly correlated with total plasma and cereal fibres intake (Aubertin-Leheudre et al., 2008).

Therefore, in the present paper we studied and evaluated the use of mass spectrometry fingerprinting to identify urinary and faecal metabolites after consumption of whole grains cereals.

2. Material and Methods

Participants and study design

A total of 30 adults aged between 25 to 53 years (mean, 37.0 ± 7.3), including 4 males and 26 females, were recruited into this study from the Ancona area, Italy. All subjects were healthy and had not received antibiotics and/or probiotics/prebiotics for at least 3 months prior to sampling. The participants were randomized to a Kamut® Khorasan (n = 15) or a whole wheat (n = 15) diet for 3 months. Each group was subdivided into 2 seasonal periods; period 1, May to July and period 2, from September to November. The study protocol was approved by the Ethics Committee of Azienda Ospedaliera Universitaria Ospedali Riuniti Umberto Lancisi e Salesi di Ancona (protocol no. 212267). Written informed consent was obtained from all participants.

Dietary food composition and sample collection

All subjects followed a nutritionally balanced but not strictly defined diet that differed excepting for the contribution of two different cereal-based foods (baked goods and pasta) whose composition is reported in Table 1 according to Simonetti et al. (2013, submitted to European Journal of Nutrition). Each participant was asked to collect a fecal and urine sample before entering the study (T0) and after 3-month dietary intervention (TF). Samples were immediately frozen at -80°C .

2.1. GC-tof-MS

GC-tof-MS analysis was carried out according to Beckmann et al.(2010). 50 μL of 0.1 mmol internal standard/L [L-threo-tert butylserine dissolved in methanol:water (70:30)] was added to 300 μL of the urine supernatant fluid. For fecal samples three methods of extraction have been used: (1) faecal water extract (water supernatant); (2) methanolic extract(100% methanol supernatant) and (3) complete extraction of methanol:water:chloroform(2:5:2). From each extracted, 200 μL of

supernatant were transferred into a 2 mL eppendorf tube containing 50 μ L of internal standard. All the samples were then dried in a speed vacuum for the derivatization. Two-step derivatization of dried samples was carried out by protecting the carbonyl moieties by methoximation with the use of 60 μ L of a 20-mg/mL solution of methoxyamine hydrochloride (Fluka) in pyridine (Fluka) at 30°C for 90 min (HTP130LP; HLC). Acidic protons were sub-sequently derivatized with 60 μ L N-methyl-N-trimethylsilyltrifluoroacetamide (Machery-Nagel GmbH) at 37°C for 30 min. Samples were allowed to cool at room temperature for 60 min. For GC-tof-MS analysis, 50 μ L supernatant fluid was transferred into 200- μ L glass vials (Chromacol), and 1 μ L was injected splitless into a Leco Pegasus III GC-tof-MS system (Leco Inc) consisting of a Focus autosampler (Anatune), an Agilent 6890N gas chromatograph equipped with a DB5-MS column (20 m x 0.25 mm internal diameter x 0.25 μ m film). The injector temperature was 250°C, the transfer line was set to 260°C, the ion source temperature was held at 230°C, and the helium flow was 1.2 mL/min. After 1 min at 80°C, the oven temperature was increased by 30°C min to 330°C, held at 330°C for 3 min, and cooled to 80°C. Automated deconvolution and peak finding were performed by using ChromaTof software (Leco). Mass spectra of all detected compounds were compared with in-house standards and spectra in the National Institute of Standards and Technology library (www.nist.gov/srd/nist1.htm) and other publicly available data-bases. All data pretreatment procedures, including baseline correction, chromatogram alignment, and data compression were performed by using custom scripts in Matlab version 6.5.1 (The Math Works Inc). Targeted peak lists were generated, and peak apex intensities of each characteristic mass in a retention time window were saved in an intensity matrix (run x metabolite).

2.2. FIE-MS

FIE-MS was performed according to Beckmann et al.(2008), Favé et al.(2011) and Lloyd et al.(2011). Aliquots of thawed urine (50 mL) were diluted in 450-mL prechilled methanol:water (3.5:1), vortex-mixed, shaken for 15 min at 4°C, and then centrifuged for 6 min at 13 000 rpm. 60 μ L of the urine and feces supernatant were put into HPLC vials and run on the ESI. Data were acquired in alternating positive and negative ionization modes and over 4 scan ranges (15–110 m/z, 100–220 m/z, 210–510 m/z, and 500–1200 m/z), with an acquisition time of 5 min, on an LTQ linear ion trap (Thermo Electron Corporation). The resulting mass spectrum was the mean of 20 scans about the apex of the infusion profile. Raw data dimensionality was reduced by electronically extracting signals with 6 0.1 Da mass accuracy and (unless shown otherwise) are presented at 1

atomic mass unit accuracy in the figures and tables. Data were \log_{10} transformed and normalized to total ion current before analysis (Beckmann et al., 2008).

2.3. Data analysis and feature selection

Data mining was carried out by following the FIEMspro workflow validated previously by Aberystwyth (Enot et al., 2008) (<http://users.aber.ac.uk/jhd/>). Principal component analysis was used to reduce data dimensionality and was followed by principal component–linear discriminant analysis (PC-LDA). Plots of the first 2 discriminant functions allowed visualization of the goodness-of-class separation, and eigenvalues (Tw) were used to evaluate the performance of PC-LDA. Discrimination was considered adequate for Tw values > 2.0 and poor for Tw values < 1.0 (21). Random forest (RF) was also used in the analysis of the multivariate data, and the RF classification margin was used to assess the classification performance (Enot et al., 2008). Feature selection techniques were used to highlight the potentially explanatory mass signals responsible for discriminating between different dietary exposure level classes. A combination of 3 methods—RF, AUC under the receiver operating characteristic curve, and Welch’s t test—were used in feature selection, to produce a full feature rank list (Enot et al., 2008). RF feature selection was performed by calculating Importance Scores, ie, the mean decrease in accuracy over all classes when a feature is omitted from the data. AUC used the AUC of the sensitivity (true-positive rate) against the specificity (false-positive rate), and the Welch’s t test ranked the features by the absolute value of the false discovery rate–corrected P values.

Randomized resampling strategies with the use of bootstrapping were applied in the process of classification and feature selection to counteract the effect of any unknown, structured variance in the data. In the current data analysis, 100 bootstraps were used for classification and feature selection with RF by using 1000 trees.

Pearson correlation coefficients between selected variables were calculated by using the function *cor* in R version 2.5.1 (Enot et al., 2008). Variables with correlation coefficients > 0.7 were considered to belong to a cluster indicative of different ionization or potential biotransformation /breakdown products of a given food-derived metabolite.

2.4. Targeted accurate mass analysis and annotation of FIE-MS signals

Selected nominal mass bins were investigated further by using targeted Nano-Flow (TriVersa NanoMate; Advion BioSciences Ltd) LTQ-Fourier transform-ion cyclotron resonance ultra-mass spectrometry (FT-ICR-MS; where ultra refers to the high-sensitivity ICR cell). Samples were prepared as for FIE-MS, but, for each urine type, 4 pools of urine samples from randomly assigned

groups of participants were prepared and reconstituted in methanol: water (80:20, vol:vol). For each spray, a sample volume of 13 mL was used, and 2 mL air was aspirated after the sample. The gas pressure was maintained between 0.2 and 0.6 psi, with the voltage at 1.4–1.7 kV (generally higher for negative ionization mode) and the current at 80–120 nA and 2100 to 260 nA in positive and negative ionization modes, respectively. Operating in narrow selected ion monitoring mode, a resolution of 100,000 was chosen and the mass range was scanned for 1 min (50–60 scans). A minimum of 3 biological replicates per class or treatment containing the specific selected mass were required for successful accurate mass verification.

The system was calibrated with LTQ-FT calibration solution prepared according to the instrument manufacturer's instructions. For metabolite signal identification, the accurate mass values were then queried by using MZedDB, an interactive accurate mass annotation tool developed by Draper et al.(2009), which can be used directly to annotate signals by means of neutral loss and/or adduct formation rules.

FIE-MSn was used for further metabolite signal identification, with the scan window set for 20 scans, an isolation width of 1 m/z, and a normalized collision energy of 40 V. An activation coefficient, Q, of 0.250 and an activation time of 30 ms were chosen, with wideband activation turned on and a source fragmentation of 20 V. The mass range settings were dependent on the molecular weight of the target ion. Chemical standards investigated with FT-ICR-MS and FIE-MSn were obtained commercially and were of HPLC grade. Standards were prepared by dissolving 1 mg of each metabolite in 1 mL extraction solvent.

3. Results

3.1. Urine data

Seasonal and urine composition differences between Kamut versus Durum can be observed using FIE-MS.

The time periods for the two intervention cohorts are in two different seasons of the year (May to July and September to November). After generating and analysing LC-MS fingerprints (FIE-MS) it is clear that seasonal diet-related signals (not from wholegrain: WG) are very different between period 1 and 2; this large variability mainly masks any impact of Kamut versus Durum on urine and fecal metabolomes. Only urine data provided some trends when combining the two seasons together (Figure 1a). We classify the data as described in the class structure of Figure 1a and made a PCA-LDA plot. PCA-LDA shows that the baseline are quite different between both groups and moreover the variation between kamut-baseline and kamut-end combining the 2 periods together is more

marked than the durum wheat variation. Therefore, we look at the 100 m/z windows of the FIE-MS spectra (in negative and positive ionisation mode, 15 m/z – 1600 m/z), only one comparison (class2 vs 4) in one particular mass range which contained some information suitable for further investigation. By comparing the impact of ‘seasons’ on urine composition, ignoring grain consumption aspects of diet, huge differences were observed (Figure 1a) in the metabolite patterns before and after dietary exposure of whole grains along the axis of maximum discrimination (DF1) with better clustering in negative ionization mode(700-800 m/z). Along the DF2 axis we see a good separation between kamut-baseline and wheat-baseline.

By splitting the periods and dividing the data into 8 class structure, we observed that from the same time period, both the base line and treatment samples as well as Kamut versus Durum interventions can be discriminated on urine metabolome, but variations are relatively weak. However, definite trends can be observed (Figure 3a,b and c). In both the PC-LDA of positive mode and negative we observe a good separation along the axis even though in the positive mode the discrimination is less obvious. By looking at 100 m/z windows of the FIE-MS spectra (in negative and positive ionisation mode, 15 m/z – 1600 m/z), only one pair-wise comparison for kamut group at period 1(2 vs 4) contained adequate information in two regions (decided by looking at pair-wise Accuracy (ACC), Area under the ROC (receiver operating characteristic) Curve (AUC) and Random Forest (RF) margin values) deserving further investigation. For the positive mode (400-500 m/z), ACC was 0.66, AUC was 0.79 and RF= 0.105, whereas for negative mode (700-800 m/z), they were 0.72, 0.91 and 0.153 respectively. From these results we selected the m/z which have the important RF scores and evaluated their relationship. Variables with correlation coefficients > 0.7 were considered to belong to a cluster indicative of different ionisation, or potential biotransformation/breakdown products of the same metabolite.

Two pairs m/z signal of the negative mode resulted correlated : m/z 754.81 with 706.72 and m/z 788.71 and 760.72 (both highly ranked) (Fig.2b). These two highly ranked signals (m/z 788.71 and 760.72) have a difference of m/z 28 which could possibly be CH_2CH_2 or CO . Fig.2.c represents the boxplot of these signals, we observe an increase in kamut for the m/z 760.72 signal while the wheat remain quiet the same. On the other hand, at m/z 788.71 signal, durum wheat decrease whereas kamut increase.

On the positive mode, it appears that 4 pairs m/z signal have a relationship (fig.2d) and we made a box plot only for the top two ranked signals (m/z 484.81 and 455.81) which have a difference of m/z 29, which could possibly be CH_2NH , CH_3CH_2 or CHO . In these box plots (Fig.2e) the trend is

similar to the negative mode as the direction of both whole grain is opposite, as kamut increase wheat durum decrease.

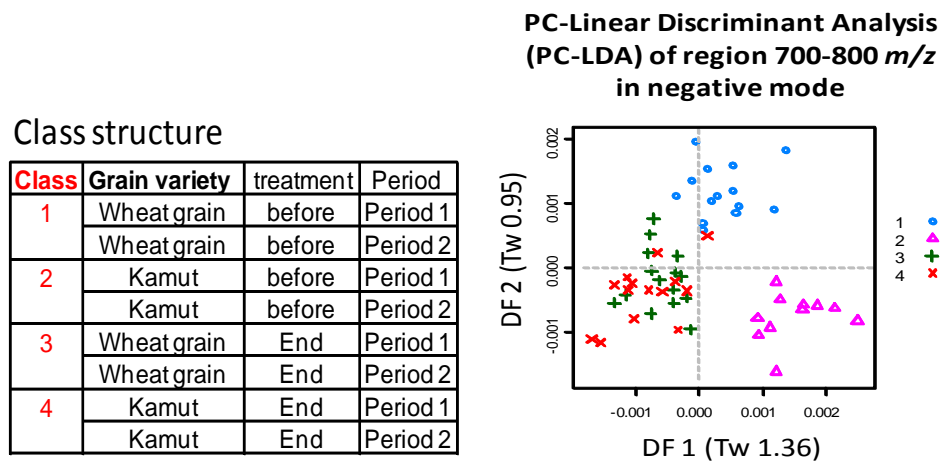


Figure 1.a. Class structure and PCA-LDA pair-wise discrimination between **treatment groups** combining the two periods and between a treatment and its corresponding baseline.

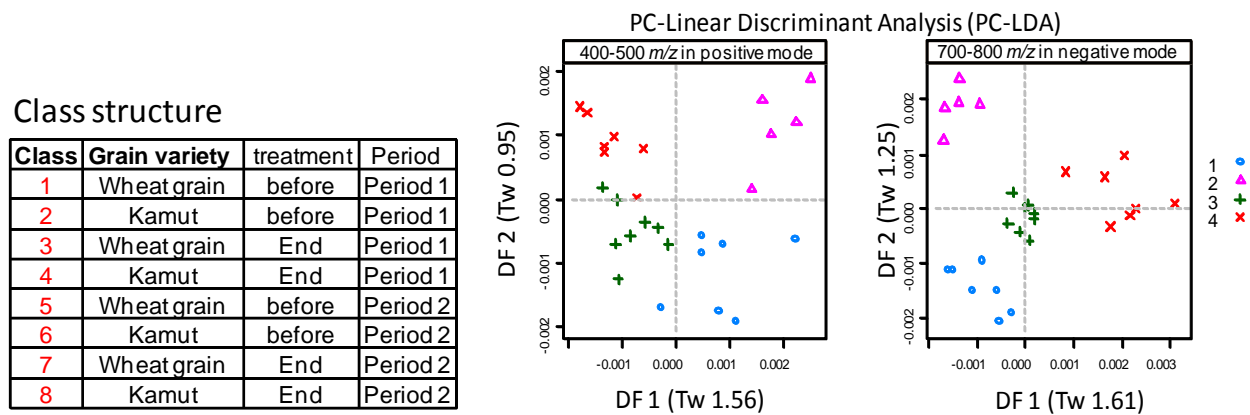


Fig 2.a. Individual seasons-pair-wise discrimination between treatment groups in each period and between treatment and its corresponding baseline

Feature selection between 2 vs 4 using a combination of RF importance scores, AUC and p-values (RF IS >0.002, P-value <0.05). Ranked according to RF IS.

RF rank	m/z	RF IS	AUC	P-value	P-value adj
1	n757.36	0.0195	1.00	0.006	0.333
2	n761.72	0.0179	1.00	0.002	0.215
3	n788.72	0.0126	0.98	0.041	0.507
4	n780.72	0.0122	0.97	0.010	0.339
5	n743.72	0.0107	0.97	0.025	0.507
6	n760.72	0.0093	0.96	0.043	0.507
7	n754.81	0.0061	0.87	0.034	0.507
8	n724.72	0.0053	0.85	0.031	0.507
9	n769.9	0.0047	0.85	0.015	0.403
10	n706.72	0.0045	0.87	0.049	0.522

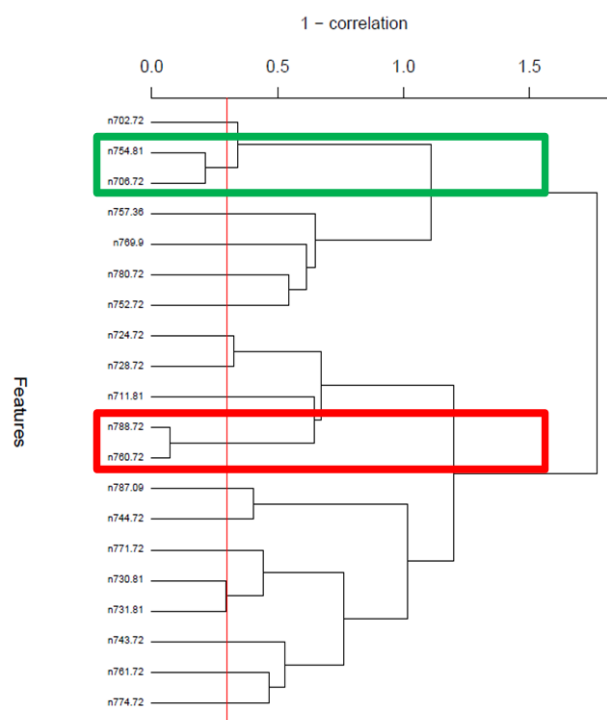


Fig 2.b. Feature selection between 2 vs 4 using a combination of RF importance scores, AUC and p-values (RF IS >0.002, P-value <0.05). Ranked according to RF IS.

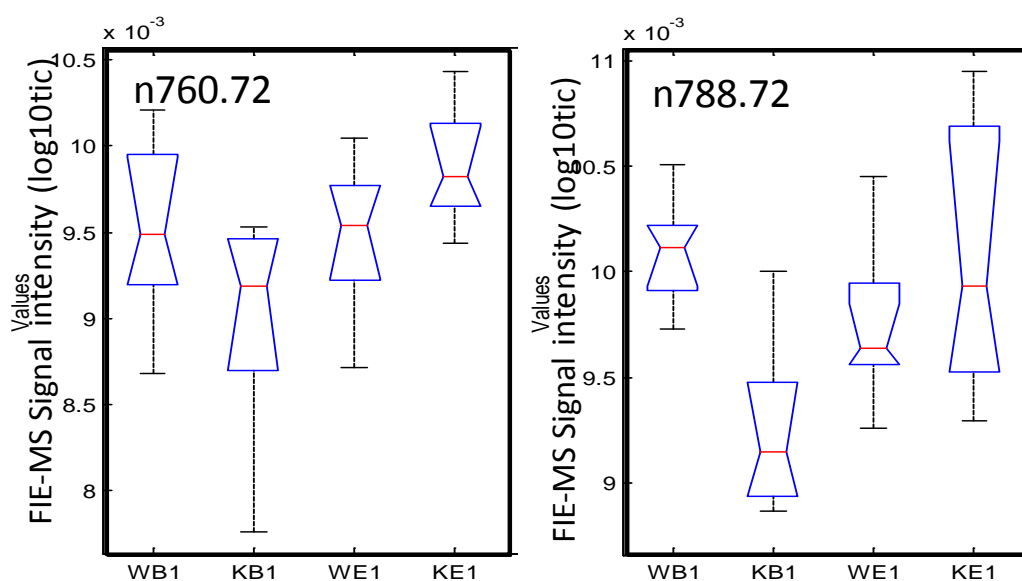


Fig. 2.c. Box plots of the top explanatory metabolite negative signals in nominal mass urine fingerprints which appear to increase after Kamut intervention in period 1. B = Before, E = end, W = wheat, K = Kamut

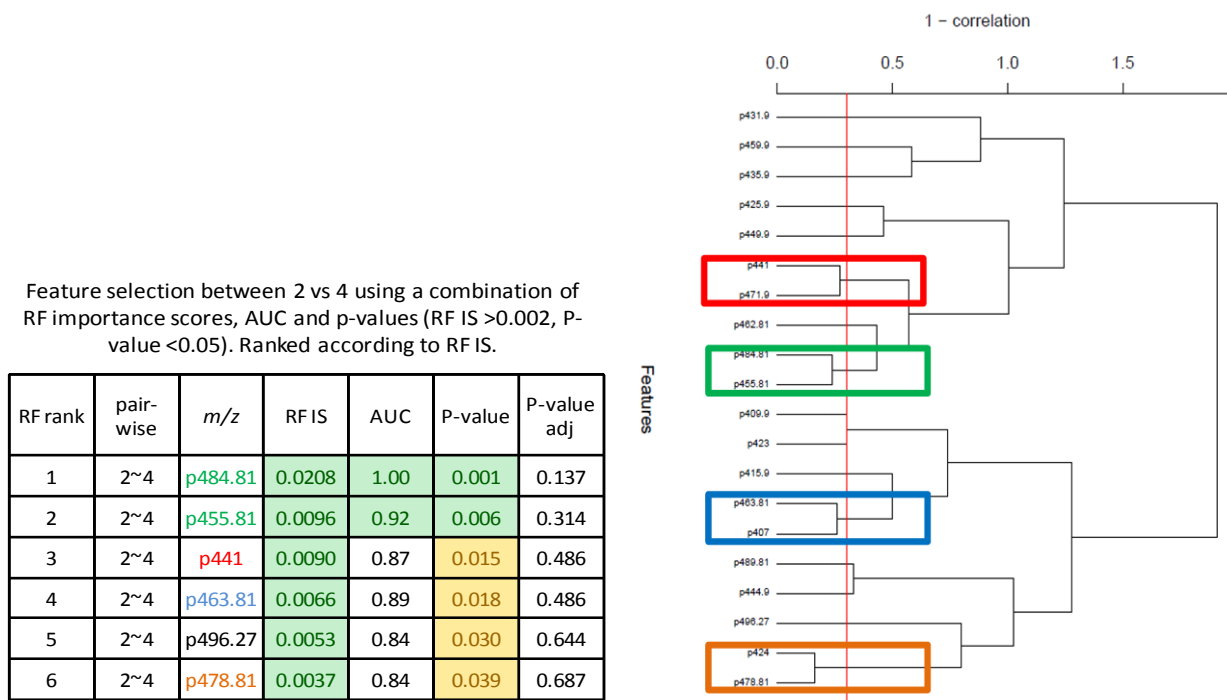


Figure. 3d. Individual seasons - urine data - positive mode features

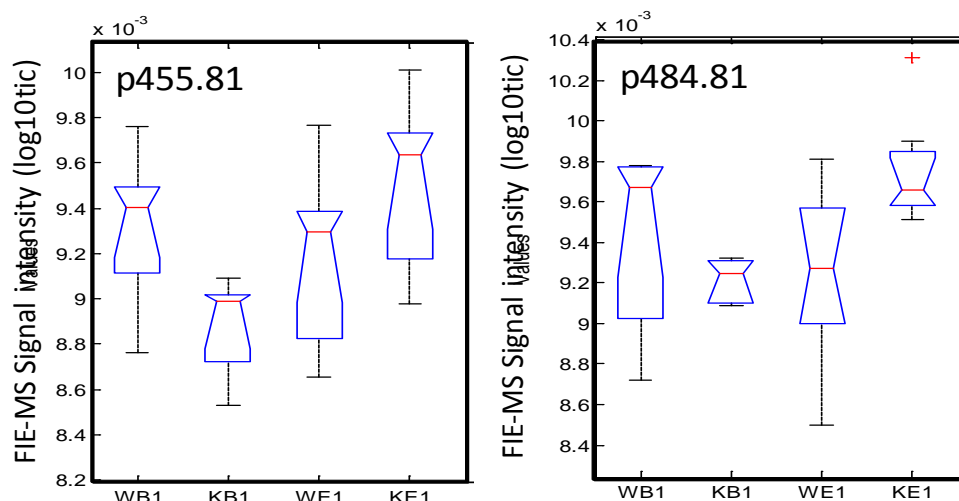


Figure. 2e. Box plots of the top explanatory metabolite positive signals in nominal mass urine fingerprints which appear to increase after Kamut exposure. B = Before, E = end, W = wheat, K = Kamut

Accurate mass analysis of urinary features that may have value as biomarkers of exposure to Kamut-containing foods

After feature selection, we evaluated if nominal mass signals previously found could be possible biomarkers of exposure to Kamut-containing foods. Only positive mode data showed better results as negative ion signals > 700 m/z were too weak. Accurate mass data and MS/MS analysis show

that m/z 484.81 and 455.81 could possibly be glucuronides which presence is usually from dietary origin (Fig.3)

RF rank	m/z	Accurate mass using FT-ICR-MS	Putative identification in positive ion data using MS/MS fragmentation patterns and MZedDB molecular formula generator
1	p484.81	485.07432	Glucuronide – loss of 176 (aglycone m/z 309.04278 – no hits
2	p455.81	455.33572	Glucuronide – loss of 176 (aglycone m/z 279.30408 – no hits
3	p441	441.04462	Unknown
		441.29878	$C_{12}H_{25}S$ & $[2M+K]^{1+} = C_{24}H_{50}S_2K$
4	p463.81	463.31492	Neither a glucuronide or sulphate conjugation
		463.32170	
		463.94781	
5	p496.27	495.89664	No MS/MS available
6	p478.81	479.00576	No MS/MS available

Figure 3. Accurate mass analysis by Fourier-Transform Ion Cyclotron Resonance Mass Spectroscopy Ultra (FT-ICR-MS) and tandem MS

Baseline level of wholegrain food appears to be highly variable between individuals.

Without a substantial washout period the baseline level of wholegrain food before the intervention appears to be highly variable between individuals. This was estimated by measuring semi-quantitatively alkylresorcinol breakdown products by GC-tof-MS in urine (3,5-dihydroxybenzoic acid [DHBA, Figure 4a] and 3-(3,5-dihydroxyphenyl)-1-propanoic acid [DHPPA, Figure 4b] . These molecules are considered as biomarkers of whole grain diet. Observing the boxplots of DHBA (Figure 4a), of the two periods joint together we saw that there is a difference between the median baseline and the median end of wheat group whereas in kamut this difference is lesser. We therefore splitted the two periods to look at the contribution and variability of each one, it resulted that there was a high variability not only among the individuals but even among each group. It appears that the period 2 showed a better effect of whole grain diet especially for the kamut group. Similar trend was found for DHPPA even though the end-baseline difference was minor in each group.

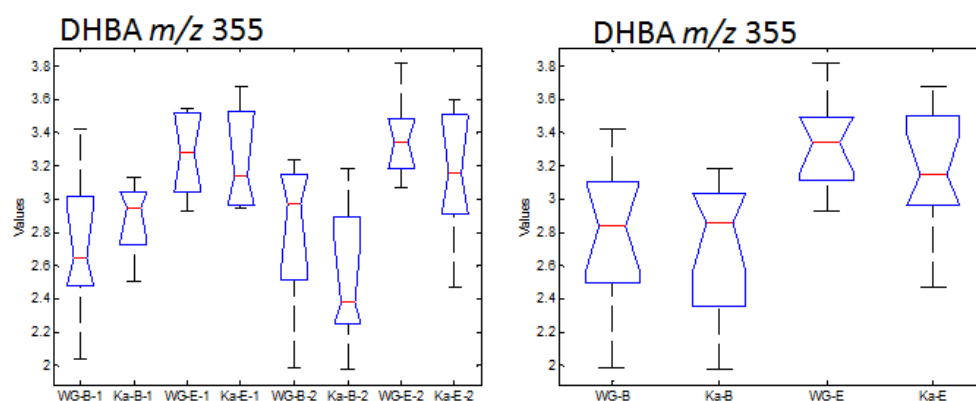


Fig 4.a. Box plots of DHBA. W = wheat; K = kamut; B = baseline; E = end; 1 = season 1; 2 = season 2; 1&2 = season 1 and 2 together

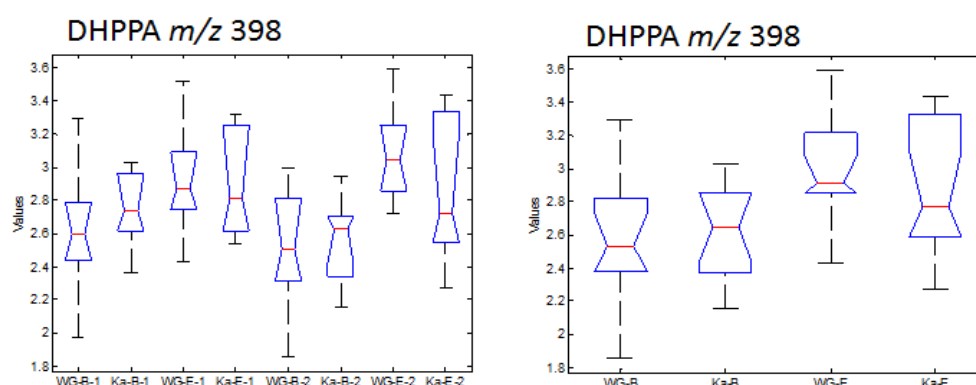


Fig 4.b. Box plots of DHPPA. W = wheat; K = kamut; B = baseline; E = end; 1 = season 1; 2 = season 2; 1&2 = season 1 and 2 together

3.2. Analysis of faecal metabolomes

The analysis of fecal (but not stool water) metabolomes by FIE-MS produced weak discrimination of Kamut versus Durum exposure but between the wheat grain group the discrimination is strong at period 1. (Figures 5a and 5b. Accurate mass analysis has not been done on these samples due to the weaker modelling characteristics than the urine dataset. Using a feature selection between pair-wise 1 vs 3 and 3 vs 4, we obtain RF rank score find at negative mode signals m/z 100-200 (Fig.5.).

Class structure

Class	Grain variety	treatment	Period
1	Wheat grain	before	Period 1
2	Kamut	before	Period 1
3	Wheat grain	End	Period 1
4	Kamut	End	Period 1
5	Wheat grain	before	Period 2
6	Kamut	before	Period 2
7	Wheat grain	End	Period 2
8	Kamut	End	Period 2

PC-Linear Discriminant Analysis (PC-LDA)

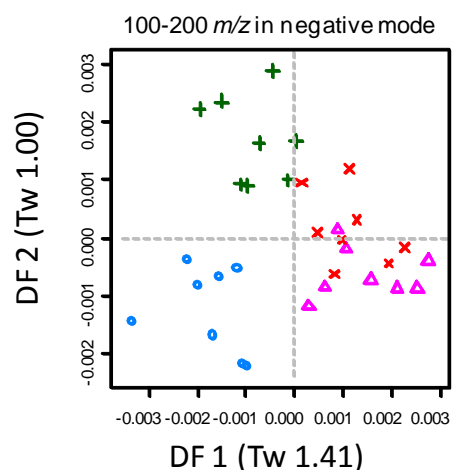


Fig 5.a. Individual seasons-pair-wise discrimination between treatment groups in each period and between treatment and its corresponding baseline in faecal samples

Modelling scores

	ACC	AUC	RF margin
Negative mode 100-200 m/z 1~3	0.74	0.86	0.124
Negative mode 100-200 m/z 3~4	0.74	0.87	0.124

RFrank	pair-wise	m/z	RFIS	AUC	P-value	P-value adj
1	n2_1~3	n116.18	0.0150	0.92	0.004	0.221
2	n2_1~3	n164.18	0.0149	0.96	0.001	0.121
3	n2_1~3	n107.63	0.0143	0.88	0.007	0.242
4	n2_1~3	n152.09	0.0141	0.92	0.035	0.319
5	n2_1~3	n108.09	0.0098	0.88	0.014	0.269
6	n2_1~3	n168.09	0.0073	0.82	0.039	0.325
7	n2_1~3	n130.18	0.0073	0.87	0.013	0.269
8	n2_1~3	n176.09	0.0068	0.84	0.016	0.269
9	n2_1~3	n117.09	0.0068	0.82	0.028	0.298
10	n2_1~3	n158.18	0.0064	0.82	0.025	0.298
11	n2_1~3	n185.18	0.0063	0.81	0.028	0.298
12	n2_1~3	n160.18	0.0056	0.82	0.029	0.298
13	n2_1~3	n146.18	0.0034	0.78	0.047	0.364
1	n2_3~4	n126.09	0.0398	1.00	0.000	0.018
2	n2_3~4	n156.18	0.0188	0.87	0.010	0.507
3	n2_3~4	n175.18	0.0136	0.84	0.039	0.819
4	n2_3~4	n125.09	0.0105	0.87	0.016	0.522

Fig 5.b. Individual seasons - faecal samples data - negative mode features

Further GC-tof-MS profiling

Comparison of aligned GC-MS chromatograms has shown that Kamut exposure can be discriminated from Wheat exposure. A very noticeable effect is a strong reduction in stool water in the level of N-acetyl-glucosamine as well as a yet still unidentified disaccharide-like molecule (Fig 6.a). Similarly, a metabolite tentatively assigned as 3-methyl-hexanedioic acid, in the faecal water (aqueous extract, Figure 6b) shows the same trend as some other metabolites in all three extracts.

Additionally to the urine biomarkers for WG consumption we have found alkylresorcinols in the third type of extract from faecal samples where only one (AR-C23) appeared discriminatory (Fig 6.c). Finally, **Figures 6d-g** show the discrimination of treatment groups by intervention using discriminant analysis, PC-LDA. Whereas we see only trends in the discrimination between classes in urine data, the strongest variance in faecal samples is again attributed most likely to seasonal diet differences.

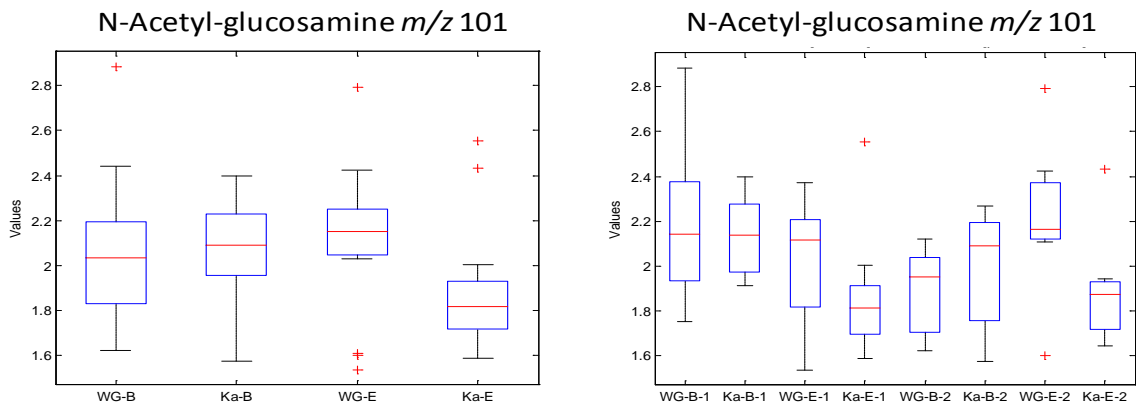


Figure 6a Methanol extract W = wheat; K = kamut; B = baseline; E = end; 1 = season 1; 2 = season 2; 1&2 = season 1 and 2 together

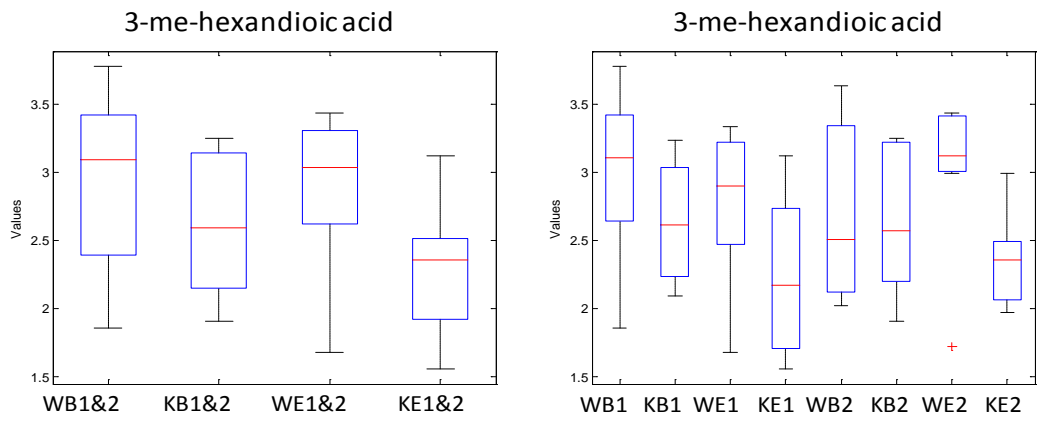


Figure 6b Faecal water

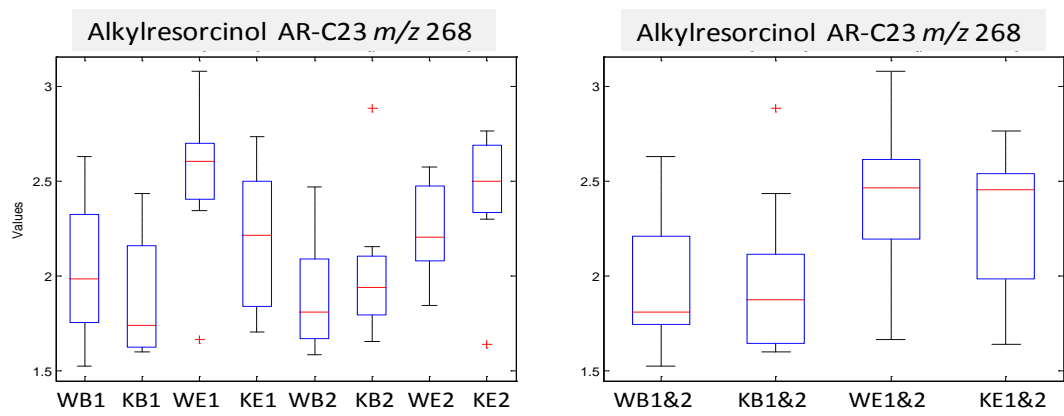
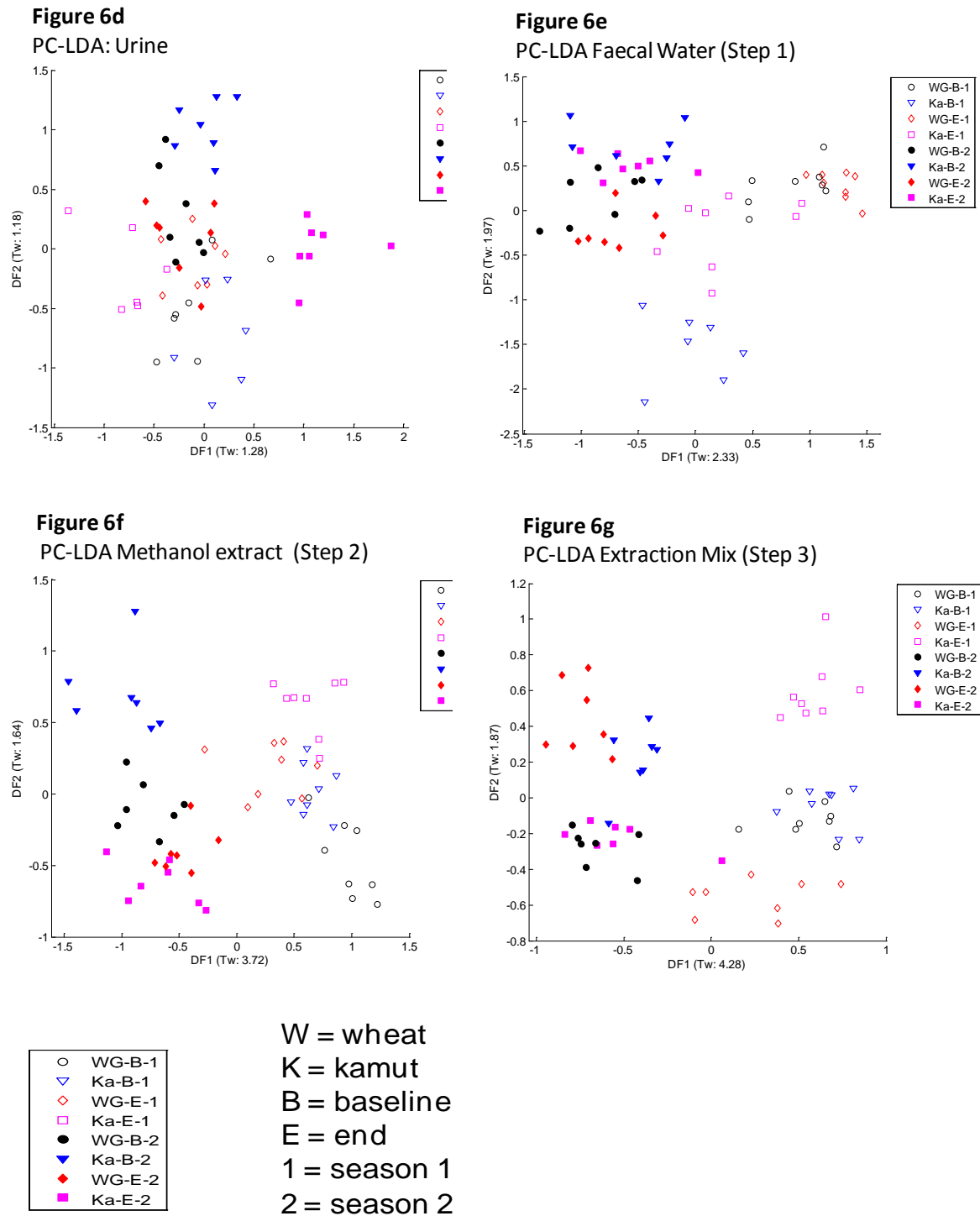


Figure 6c. Alkylresorcinols only in extracts using Extraction Mix (CHCl₃/MeOH/H₂O 2/5/2)



4. Discussion

This study highlighted the importance of design of experiment prior to a diet intervention specially if we want to evaluate the effect of a specific diet on the humans metaboloma. Firstly, the absence of a substantial washout period before the intervention leads to huge variability between individuals at the baseline level of wholegrain foods. Crossover studies such as run-in diets before the

intervention (Darmaun et al., 2011) or wash-out periods (Xie et al., 2012) are frequent trial design strategies aimed to reduce the baseline variation in human dietary intervention studies; however the optimal amount of time for the normalization period is not well understood. A run-in diet can start at short-term, transient metabolic response and confound the biological mechanisms under examination. Moreover, the variation by which individuals are uniquely affected by a run-in diet is not well understood. These study parameters deserve methodical, focused research to better differentiate fluctuations in metabolic status during different life stages (Darmaun et al., 2011).

Secondly, we also found high variation between the two periods meaning that multivariate modelling can only be done with samples collected in the same time period, reducing the number of replicates by 50% (only 7-8 per class) which is not always sufficient to obtain adequate statistical outcomes with the degree of initial variability of WG intake. Many metabolomics studies in mammals have highlighted the importance of inter- and intra-individual metabolite variation (Lenz et al., 2003; Colyer et al., 2011; Gruden et al., 2012). In nutritional metabolomics study, the biochemical properties and composition of cells and tissues make difficult the analysis, and also because the metabolite profile is only a snapshot of metabolism at a given time (Krug et al., 2012). Metabolite variations are influenced by many factors such as diet history and environmental exposures, presence and gravity of infections or chronic diseases, and genetics (Gruden et al., 2012). All these factors can contribute to individual metabolite variation which is inherently difficult to control.

Comparing the two treatments and their respective baseline, we realise that the intervention was not specifically designed for metabolomics analysis because of the weak differences observed, it seemed that some of these participants were already whole grain consumers at the beginning of the intervention. This is compared to a previous study done by Lloyd et al. (2013), where they implemented a 4 week washout period prior to an intervention study where individuals consumed 3 servings a day of wholegrain wheat or rye products for 4 weeks, and for the subsequent 4 weeks these individuals consumed 6 servings/day.

Even though, GC-tof-MS profiling can generally expose molecules that are not particularly well ionised during FIE-MS, we have been able to develop GC-MS chromatograms and measure two targeted molecules in urine samples: DHBA and DHPPA, the variation of these biomarkers was not good enough compared to others studies (Aubertin-Leheudre et al., 2008). The reduction of N-acetyl-glucosamine and 3-methyl-hexanedioic acid in kamut faecal samples suggests that Kamut can have a role in modulating mucus production/degradation or even gut inflammation. This theory has also been reported by Sofi et al. (2013) who concluded that a high intake diet of kamut

products might reduce markers of both oxidative stress and inflammatory status, as well as metabolic risk factors. For example, several studies reported that consumption of cereals fructans is correlated to a reduction in production of pro-inflammatory biomarkers, simultaneously with an increase of gut bifidobacteria and lactobacilli (Videla et al., 2001; Welters et al., 2002). Fecal biomarkers have shown promising results in differentiating inflammatory bowel disease IBD from IBS irritable bowel syndrome, monitoring disease activity and predicting the relapse in IBD (D’Inca et al., 2008; Konikoff & Denson, 2006).

Finally our results in faecal samples showed that method extraction is very important for metabolomics analysis as we saw a different distribution of the data set according to the discrimination analysis. The dietary intakes and responses are related to the sample used for the analysis, so using blood plasma, serum, urine, stool, saliva, muscle, and liver metabolomes can give different results, different aspects of dietary intakes and responses (Walsh et al., 2006; Kleemann et al., 2007, Pettersson et al., 2008). In general, urine and/or stool may be more appropriate to evaluate degradation or detoxification pathways. Moreover, the fecal metabolome can offer more informations into the response of the microbiome to nutritional interventions (Gill et al., 2010; Pettersson et al., 2008).

References

- Alexandre-Gouabau, M. C.; Courant, F.; Le Gall, G.; Moyon, T.; Aubertin-Leheudre M, Koskela A, Marjamaa A, Adlercreutz H.(2008). Plasma alkylresorcinols and urinary alkylresorcinol metabolites as biomarkers of cereal fiber intake in finnish women. *Cancer Epidemiol Biomarkers Prev* 17:2244–2248.
- Beckmann M, Enot DP, Overy DP, Scott IM, Jones PG, Allaway D, Draper J.(2010).Metabolite fingerprinting of urine suggests breed-specific dietary metabolism differences in domestic dogs. *Br J Nutr* 103:1127–38.
- Beckmann M, Parker D, Enot DP, Duval E, Draper J.(2008). High-throughput, nontargeted metabolite fingerprinting using nominal mass flow injection electrospray mass spectrometry. *Nat Protoc* 3:486–504
- Colyer, A.; Gilham, M.S.; Kamlage, B.; Rein, D.; Allaway, D.(2011). Identification of intra- and inter-individual metabolite variation in plasma metabolite profiles of cats and dogs. *Br. J. Nutr.* 106 Suppl 1: S146-149.
- D’Inca R, Dal Pont E, Di Leo V, Benazzato L, Martinato M, et al. (2008). Can calprotectin predict

- relapse risk in inflammatory bowel disease? *Am J Gastroenterol* 103: 2007–14.
- Darmaun, D.; Parnet, P.; Coupe, B.; Antignac, J.P.(2011). Offspring metabolomic response to maternal protein restriction in a rat model of intrauterine growth restriction (IUGR). *Journal of proteome research* 10, 3292-3302.
- Draper J, Enot DP, Parker D, Beckmann M, Snowdon S, Lin WC,Zubair H.(2009). Metabolite signal identification in accurate mass metabolomics data with MZedDB, an interactive m/z annotation tool utilising predicted ionisation behaviour 'rules.' *Bmc Bioinf* 10.
- Enot DP, Lin W, Beckmann M, Parker D, Overy DP, Draper J.(2008). Preprocessing, classification modeling and feature selection using flow injection electrospray mass spectrometry metabolite fingerprint data. *Nat Protoc* 3:446–70.
- Fave G, Beckmann ME, Draper J et al. (2009) Measurement of dietary exposure: a challenging problem which may be overcome thanks to metabolomics? *Genes Nutr* 4, 135–141.
- Favé G, Beckmann ME, Lloyd AJ, Zhou S, Harold G, Lin W, Tailliar K, Draper JH, Mathers JC.(2011). Development and validation of a standardized protocol to monitor human dietary exposure by metabolite fingerprinting of urine samples. *Metabolomics* 7:469–84.
- Gianotti, A., Danesi, F., Verardo, V., Serrazanetti, D. I., Valli, V., Russo, A., Riciputi, Y., Tossani, N., Caboni, M. F., Guerzoni, M. E., & Bordonì, A. (2011). Role of cereal type and processing in whole grain in vivo protection from oxidative stress. *Frontiers in Bioscience (Landmark Ed)*, 16, 1609-1618
- Gill, C.I.; McDougall, G.J.; Glidewell, S.; Stewart, D.; Shen, Q.; Tuohy, K.; Dobbin, A.; Boyd, A.; Brown, E.; Haldar, S.; Rowland I. R. Profiling of phenols in human fecal water after raspberry supplementation. *J. Agric. Food. Chem.*, 2010, 58, 10389-10395.
- Gruden, K.; Hren, M.; Herman, A.; Blejec, A.; Albrecht, T.; Selbig, J.; Bauer, C.; Schuchardt, J.; Or-Guil, M.; Zupancic, K.; Svajger, U.; Stabuc, B.; Ihan, A.; Kopitar, A. N.; Ravnikar, M.; Knezevic, M.; Rozman, P.; Jeras, M. (2012). A "crossomics" study analysing variability of different components in peripheral blood of healthy caucasoid individuals. *PloS one*, 7, e 28761.
- Hardin DS.(2009). Validating dietary intake with biochemical markers. *J Am Diet Assoc* 109:1698–1699.
- Institute of Medicine of the National Academies: Dietary reference intakes.(2007), research synthesis workshop summary. Washington, DC: The National Academies Press.
- Jenab M, Slimani N, Bictash M et al. (2009) Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum Genet* 125, 507–525.
- Jenab M, Slimani N, Bictash M, Ferrari P, Bingham S.(2009). Biomarkers in nutritional

epidemiology: applications, needs and new horizons. *Hum Genet* 125:507–525.

- Kleemann, R.; Verschuren, L.; van Erk, M. J.; Nikolsky, Y.; Cnubben, N.H.; Verheij, E. R.; Smilde, A.K. Hendriks, H. F.; Zadelaar, S.; Smith, G.J.; Kaznacheev, V.; Nikolskaya, T.; Melnikov, A.; Hurt-Camejo, E.; van der Greef, J.; van Ommen, B.; Kooistra T.(2007) Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis. *Genome Biol* 8, R200.
- Konikoff MR, Denson LA .(2006). Role of faecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. *Inflamm Bowel Dis* 12: 524–34.
- Krug, S., Kastenmuller, G.; Stuckler, F.; Rist, M. J.; Skurk, T.; Sailer, M.; Raffler, J.; Romisch-Margl, W.; Adamski, J.; Prehn, C.; Frank, T.; Engel, K.H.; Hofmann, T.; Luy, B.; Zimmermann, R.; Moritz, F.; Schmitt-Kopplin, P.; Krumsiek, J.; Kremer, W.; Huber, F.; Oeh, U.; Theis, F. J.; Szymczak, W.; Hauner, H.; Suhre, K.; Daniel. H. (2012). The dynamic range of the human metabolome revealed by challenges. *FASEB J.* 26, 2607-2619
- Lenz, E. M.; Bright, J.; Wilson, I. D.; Morgan, S.R.; Nash A.F. (2003). A ¹H NMR-based metabonomic study of urine and plasma samples obtained from healthy human subjects. *J. Pharm. Biomed. Anal.*, 33, 1103-1115.
- Linko-Parvinen A-M, Landberg R, Tikkanen MJ, Adlercreutz H, Peñalvo JL.(2007). Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. *J Nutr* 137:1137–1142.
- Lloyd AJ, Beckmann M, Haldar S et al. (2013) Data-driven strategy for the discovery of potential urinary biomarkers of habitual dietary exposure. *Am J Clin Nutr* 97, 377–389.
- Lloyd AJ, Favé G, Beckmann M, Lin W, Tailliant K, Xie L, Mathers JC, Draper J.(2011). Use of mass spectrometry fingerprinting to identify urinary metabolites after consumption of specific foods. *Am J Clin Nutr* 2011;94:981–91.
- McCabe-Sellers B: Advancing the art and science of dietary assessment through technology. *J Am Diet Assoc* 2010, 110:52–54.
- Mennen LI, Sapinho D, Ito H et al. (2006) Urinary flavonoids and phenolic acids as biomarkers of intake for polyphenolrich foods. *Br J Nutr* 96, 191–198.
- Monsen ER.(2003). *Research Successful Approaches*. 2nd edition. Chicago, IL: American Dietetic Association :1–482.
- Penn L, Boeing H, Boushey CJ et al. (2010) Assessment of dietary intake: NuGO symposium report. *Genes Nutr* 5, 205–213.
- Pettersson, J.; Karlsson, P. C.; Choi, Y. H.; Verpoorte, R.; Rafter, J.J.; Bohlin L. (2008). NMR

- metabolomic analysis of fecal water from subjects on a vegetarian diet. *Biol Pharm Bull* 31, 1192-1198.
- Potischman N.(2003). Biologic and methodologic issues for nutritional biomarkers. *J Nutr* 133:875S–880S.
- Rasmussen LG, Winning H, Savorani F et al. (2012) Assessment of the effect of high or low protein diet on the human urine metabolome as measured by NMR. *Nutrients* 4, 112–131.
- Scalbert A, Brennan L, Fiehn O et al. (2009) Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. *Metabolomics* 5, 435–458.
- Simonato, B., Pasini, G., Giannattasio, M., & Curioni, A. (2002). Allergenic potential of Kamut wheat. *Allergy*, 57, 653-654.
- Sofi, F., Whittaker, A., Cesari, F., Gori, A. M., Fiorillo, C., Becatti, M., Marotti, I., Dinelli, G., Casini, A., Abbate, R., Gensini, G. F., & Benedettelli, S. (2013). Characterization of Khorasan wheat (Kamut) and impact of a replacement diet on cardiovascular risk factors: cross-over dietary intervention study. *European Journal of Clinical Nutrition*, 67, 190-195.
- Stella C, Beckwith-Hall B, Cloarec O et al. (2006) Susceptibility of human metabolic phenotypes to dietary modulation. *J Proteome Res* 5, 2780–2788
- Thompson FE, Subar AF, Loria CM, Reedy JL, Baranowski T.(2010). Need for technological innovation in dietary assessment. *J Am Diet Assoc* 110:48–51.
- Videla S, Vilaseca J, Antolin M, Garcia-Lafuente A, Guarner F, Crespo E, et al.(2001). Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. *Am J Gastroenterol* 96:1486–93
- Walsh, M. C.; Brennan, L. Malthouse, J. P.; Roche, H. M.; Gibney, M. J.(2006). Effect of acute dietary standardization on the urinary, plasma, and salivary metabolomic profiles of healthy humans. *Am. J. Clin Nutr* 84, 531-539.
- Welters CF, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG.(2002). Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. *Dis Colon Rectum* 45:621–7.
- Xie, G.; Zhao, A.; Zhao, L.; Chen, T.; Chen, H.; Qi, X.; Zheng, X.; Ni, Y.; Cheng, Y.; Lan, K.; Yao, C.; Qiu, M.; Jia, W.(2012). Metabolic Fate of Tea Polyphenols in Humans. *J. Proteome. Res.*

GENERAL CONCLUSIONS

The use of immature cereal grains can be a validated alternative to have cereals products with high content of dietary fibre or phenolic compounds.

Using wheat flour at fully ripe stage during industrial fermentation can increase the content of resistant starch up to 4.9 g/100g applying high temperature in the baked products while using kamut flour at fully ripe during industrial fermentation at low temperature the fructans amount can reach a level of 2.1g/100g.

To enhance the polyphenols and flavonoids content is better to use sourdough fermentation, in particular when the flour is at milky stage. In fact, high levels of flavonoids (52.89 mg CE/100g) and polyphenols (324.57 mg GAE) were obtained using wheat flour at milky stage during sourdough fermentation at low temperature.

From the metabolomic analyses it resulted that a high intake of kamut products can probably reduce markers of both oxidative stress and inflammatory status, and also metabolic risk factors. So further investigations should be done on these effects.

This study also highlighted the importance of design of experiment prior to a diet intervention especially for the evaluation of the effect of a specific diet on the human's metaboloma. The dietary intakes and responses are related to the sample used for the analysis, so different biological samples can lead to different results, different aspects of dietary intakes and responses.